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<b>(21) International Application Number:</b> PCT/DK00/00025 <b>(22) International Filing Date:</b> 21 January 2000 (21.01.00)  <b>(30) Priority Data:</b> PA 1999 00092      22 January 1999 (22.01.99)      DK PA 1999 01340      21 September 1999 (21.09.99)      DK  <b>(71) Applicant:</b> NOVO NORDISK A/S [DK/DK]; Enzyme Business Patents, Novo Allé, DK-2880 Bagsværd (DK).  <b>(72) Inventor:</b> LEHMANN, Martin; 258 Sayre Drive, Princeton, NJ 07043 (US).		<b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>With amended claims.</i>
<b>(54) Title:</b> IMPROVED PHYTASES  <b>(57) Abstract</b>  <p>This invention relates to improved phytases, preferably phytases of an increased thermostability, and a process of producing them. In particular, stabilizing amino acid mutations are introduced into a homologous protein, or the active site of a phytase is replaced in part or in total. The corresponding DNA sequences and methods of preparing them are also disclosed, as are methods of producing the improved phytases, and the use thereof. Specific variants of <i>Aspergillus fumigatus</i> phytase and of consensus phytases are disclosed.</p>		

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### Improved phytases

Phytases are enzymes that hydrolyze phytate (myo-inositol hexakisphosphate) to myo-inositol and inorganic phosphate. They  
5 are known to be valuable feed additives.

The present invention relates to improved phytases, viz. phytases of amended characteristics, e.g. amended activity characteristics, reference being made to e.g. the phytase(s) it has been derived from, or to known phytases. Amended activity  
10 characteristics means amended in at least one phytase activity related respect, such as (non-exclusive list): pH stability, temperature stability, pH profile, temperature profile, specific activity (in particular in relation to pH and temperature), substrate specificity, substrate cleavage pattern, substrate  
15 binding, position specificity, the velocity and level of release of phosphate from corn, reaction rate, phytate degradation rate), end level of released phosphate reached.

Examples of amended activity characteristics are amended specific activity (e.g. increased, e.g. increased at a pH of 3,  
20 4, 5, or 6); amended pH or temperature profile; and/or amended (e.g. increased) thermostability, e.g. of an increased melting temperature as measured using Differential Scanning Calorimetry (DSC).

The present invention also relates to a process for the  
25 preparation of a modified protein, wherein in a first step a consensus sequence is determined from a number of highly homologous sequences according to steps a), b) and c) below:

a) at least three, preferably at least four amino acid sequences are aligned by any standard alignment program known in  
30 the art;

b) at every position of the amino acid sequence alignment, the amino acids are evaluated for their evolutionary similarity and a consensus residue is chosen by any standard program known in the art, whereby the minimal requirements for calculation of  
5 a consensus residue are set in such a way that the program is already able to determine a consensus residue if a given residue occurs in only two of the aligned sequences. However, if there is a subgroup of sequences among the compared amino acid sequences that shows a much higher degree of similarity with  
10 each other than with the remaining sequences of the alignment, the subgroup may be represented in the calculation only with its consensus sequence determined in the same way as outlined in EP 897985, or alternatively, to each sequence of the subgroup, a vote weight of 1 divided by the number of sequences in the  
15 subgroup will be assigned;

c) in case no consensus amino acid at a defined position is identified by the program, any of the amino acids, preferably the most frequently occurring amino acid at this position is selected.

20 In a second aspect of the invention, a homologous sequence is compared with the consensus sequence, and one or more non-consensus residues in this homologous sequence are replaced by the corresponding consensus residues.

Preferably, only such amino acid residues are replaced in  
25 the homologous amino acid sequence where a consensus residue can clearly be defined by the program under moderately stringent conditions whereas at all positions of the alignment where no preferred consensus amino acid can be determined under moderately stringent conditions, the amino acids of the  
30 homologous protein remain unchanged.

In a third aspect of the invention, the active center of the protein of interest is determined, comprising all amino acid residues that are involved in forming the active center, both in the consensus sequence, and in the sequence of a homologous protein; subsequently, some or all of the divergent amino acid residues of the homologous protein are inserted in the backbone of the consensus sequence.

In one embodiment of this process, the program used for the comparison of amino acids at a defined position regarding their evolutionary similarity is the program "PRETTY".

The active center of the protein can be determined by using an analysis of the three-dimensional structure of the protein.

An example of a homologous protein is an enzyme family, an example of a defined protein family is the family of phytases, e.g. of fungal origin.

For example, the amino acid sequence of the phytase can be changed by the introduction of at least one mutation or substitution chosen from

20	E58A	F54Y
	D69K	I73V
	D197N	K94A
	T214L	R101A
	E222T	N153K
25	E267D	V158I
	R291I	A203G
	R329H	S205G
	S364T	V217A
	A379K	A227V
30	G404A	V234L
		P238A

Q277E  
 A287H  
 A292Q  
 V366I  
 5 A396S  
 E415Q  
 G437A  
 R451E

For interpreting these abbreviations, as an example, the  
 10 mutation E58A is to be interpreted as follows: When subtracting  
 26 from the number, you get the position or residue number in  
 the consensus phytase sequence or another phytase sequence  
 aligned as shown in Fig. 1 (corresponding to the addition of a  
 26 amino acid signal sequence to the sequences shown in Fig. 1).  
 15 For example, in E58A, number 58 means position number 32 ( $58 - 26 = 32$ ). And the letter before the number, i.e. E, represents the  
 amino acid in the phytase to be modified which is replaced by  
 the amino acid behind the number, i.e. A.

The above-mentioned amino acid replacements, alone and/or  
 20 in combination, have a positive effect on the protein stability.

The following sub-groups of mutations are also interesting  
 (i.e. phytases comprising at least one mutation selected from  
 either one of the groups of):

E58A, D69K, D197N, T214L, E222T, E267D, R291I, R329H,  
 25 S364T, A379K, G404A;

F54Y, I73V, K94A, R101A, N153K, V158I, A203G, S205G,  
 V217A, A227V, V234L, P238A, Q277E, A287H, A292Q, V366I, A396S,  
 E415Q, G437A, R451E;

E58A, D69K, D197N, F54Y, I73V, K94A;  
 30 T214L, E222T, E267DR101A, N153K, V158I;  
 R291I, R329H, S364TA203G, S205G, V217A;

A379K, G404AA227V, V234L, P238A, Q277E;  
A287H, A292Q, V366I, A396S, E415Q, G437A, R451E;  
T214L, E222T, S364T, V158I, A203G, G404A, A227V, P238A,  
A396S, G437A, R451E.

5        Examples of host cells are plant cells, animal cells, and microbial cells, e.g. prokaryotic or eukaryotic cells, such as bacterial, fungal or yeast cells. An example of a fungal host is a strain of the genus *Aspergillus*, and examples of yeast hosts are strains of *Saccharomyces*, and strains of *Hansenula*.

10        The invention also relates to a modified protein obtainable or obtained by any of the processes described above.

      The invention also relates to a variant or mutein of a phytase such as (but not limited to) the consensus phytase-1, wherein, in the amino acid sequence in Figure 2, at least one  
15 of the following replacements have been effected: Q50L, Q50T, Q50G, Q50T-Y51N, Q50L-Y51N or Q50T-K91A.

      In the third aspect mentioned above, a consensus sequence is determined from homologous sequences as described above; in a second step the active center of the protein comprising all  
20 amino acid residues that are involved in forming the active center is determined in the consensus sequence and in the sequence of a single homologous protein as well. The single homologous protein may have preferred properties like high specific activity or different pH dependency of enzymatic  
25 activity. In a third step some or all amino acid residues that are involved in forming the active center of the homologous protein are inserted into the backbone of the consensus sequence. The result thereof is a chimeric protein having the active center derived from a single protein and the backbone of  
30 the consensus sequence.

The active center of the protein can be determined e.g. by using any analysis of the three-dimensional structure of the protein, e.g. by homology modelling on the basis of a known 3D-structure of a known protein.

5 The present invention also provides consensus proteins obtainable or obtained by such processes, in particular proteins comprising at least one of the amino acid sequences shown in Figures 2-6, 10 or 21, or variants or muteins thereof. Examples of such variants are shown in Figs. 7-9.

10 Such variants or muteins can be defined and prepared on the basis of the teachings given in European Patent Application number 0897010, e.g. Q50L, Q50T, Q50G, Q50L-Y51N, or Q50T-Y51N. These mutations are defined as above, or, alternatively, by reference to Fig. 2. When referring to Fig. 2, no subtraction of  
15 the 26 amino acid signal peptide is required (e.g. in "Q50L," at position 50 of the amino acid sequence of Fig. 2, the amino acid Q has been replaced by amino acid L).

A food, feed, or pharmaceutical composition comprising the phytases of the invention is another aspect of the invention.

20 In this context, and relating to the process of the invention, "at least three, preferably at least four amino acid sequences of such defined protein family" means that three, four, five, six to twelve, twenty, fifty, or even more sequences can be used for the alignment and the comparison to create the  
25 amino acid sequence of the consensus protein. "Sequences of a defined protein family" means that such sequences fold into a three-dimensional structure, wherein the alpha-helices, the beta-sheets and beta-turns are at the same position so that such structures are, as called by the man skilled in the art, largely  
30 superimposable. Furthermore these sequences characterize proteins that show the same type of biological activity, e.g. a

defined enzyme class, e.g. the phytases. The three-dimensional structure of one such protein is sufficient to allow the modelling of the structure of the other homologous proteins of such a family. An example, how this can be done, is given in

5 Example 1. "Evolutionary similarity" in the context of the present invention refers to a scheme which classifies amino acids regarding their structural similarity which allows that one amino acid can be replaced by another amino acid with a minimal influence on the overall structure, as this is done e.g.

10 by programs, like "PRETTY", known in the art. The phrase "the degree of similarity provided by such a program...is set to less stringent number" means in the context of the present invention that values for the parameters which determine the degree of similarity in the program used in the practice of the present

15 invention are chosen in a way to allow the program to define a consensus amino acid for a maximum of positions of the whole amino acid sequence, e. g. in case of the program PRETTY a value of 2 or 3 for the THRESHOLD and a value of 2 for the PLURALITY can be chosen. Furthermore, "a vote weight of one divided by the

20 number of such sequences" means in the context of the present invention that the sequences which define a group of sequences with a higher degree of similarity as the other sequences used for the determination of the consensus sequence only contribute to such determination with a factor which is equal to one

25 divided by the number of all sequences of this group.

As mentioned before, should the program not allow to select the consensus amino acid, the most frequent amino acid is selected; should the latter be impossible the man skilled in the art will select an amino acid from all the sequences used for

30 the comparison which is known in the art for its property to improve the thermostability in proteins as discussed e.g. by

Janecek, S. (1993), *Process Biochem.* 28, 435-445; Fersht, A. R. & Serrano, L. (1993), *Curr. Opin. Struct. Biol.* 3, 75-83; Alber, T. (1989), *Annu. Rev. Biochem.* 58, 765-798; Matthews, B. W. (1987), *Biochemistry* 26, 6885-6888; or Matthews, B. W. (1991),  
5 *Curr. Opin. Struct. Biol.* 1, 17-21.

The stability of an enzyme is relevant for many industrial applications. Therefore, a lot of attempts, more or less successful, have been made to improve the stability, preferably the thermostability of enzymes by rational or random approaches.

10 Here we present an alternative way to improve the thermostability of a protein.

The invention provides a process for the preparation of a consensus protein comprising a process to calculate an amino acid residue for nearly all positions of a so-called consensus  
15 protein and to synthesize a complete gene from this sequence that can be expressed in a pro- or eukaryotic expression system.

DNA sequences of the present invention can be constructed starting from genomic or cDNA sequences encoding the proteins, e.g. phytases, of interest. For example, they can be constructed  
20 by methods of in vitro mutagenesis [see e.g. Sambrook et al., *Molecular Cloning*, Cold Spring Harbor Laboratory Press, New York]. A widely used strategy for "site-directed mutagenesis", as originally outlined by Hurchinson and Edgell [*J. Virol.* 8, 181 (1971)], involves the annealing of a synthetic  
25 oligonucleotide carrying the desired nucleotide substitution to a target region of a single-stranded DNA sequence wherein the mutation should be introduced [for review see Smith, *Annu. Rev. Genet.* 19, 423 (1985), and for improved methods, see references 2-6 in Stanssen et al., *Nucl. Acids Res.*, 17, 4441-4454 (1989).  
30 Another possibility of mutating a given DNA sequence is the mutagenesis by using the polymerase chain reaction (PCR). DNA as

starting material can be isolated by methods known in the art and described e.g. in Sambrook et al. (Molecular Cloning) from the respective strains.

For strain information, see e.g. EP 684313 or any  
5 depository authority indicated below. *Aspergillus niger* [ATCC 9142], *Myceliophthora thermophila* [ATCC 48102], *Talaromyces thermophilus* [ATCC 20186] and *Aspergillus fumigatus* [ATCC 34625] have been redeposited according to the conditions of the Budapest Treaty at the American Type Culture Cell Collection  
10 under the following accession numbers: ATCC 74337, ATCC 74340, ATCC 74338 and ATCC 74339, respectively. It is, however, understood that DNA encoding a consensus protein in accordance with the present invention can also be prepared in a synthetic manner as described, e.g. in EP 747483 or EP 897985, or in the  
15 examples, by methods known in the art.

For sequence information, see e.g. EP 684313, or sequence data bases, for example like Genbank (Intelligenetics, California, USA), European Bioinformatics Institute (Hinxton Hall, Cambridge, GB), NBRF (Georgetown University, Medical  
20 Centre, Washington DC, USA) and Vecbase (University of Wisconsin, Biotechnology Centre, Madison, Wisconsin, USA).

The process of the present invention can e.g. be used to improve the thermostability of the enzyme phytase.

Once complete DNA sequences of the present invention have  
25 been obtained they can be integrated into vectors by methods known in the art and described e.g. in Sambrook et al. (s.a.) to overexpress the encoded polypeptide in appropriate host systems. However, a man skilled in the art knows that also the DNA sequences themselves can be used to transform the suitable host  
30 systems of the invention to get overexpression of the encoded polypeptide. Appropriate host systems are for example fungi,

like Aspergilli, e.g. *Aspergillus niger* [ATCC 9142] or *Aspergillus ficuum* [NRRL 3135] or like *Trichoderma*, e.g. *Trichoderma reesei*; or yeasts, like *Saccharomyces*, e.g. *Saccharomyces cerevisiae* or *Pichia*, like *Pichia pastoris*, or  
5 *Hansenula polymorpha*, e.g. *H. polymorpha* (DSM5215); or plants, as described, e.g. by Pen et al., *Bio/Technology* 11, 811-814 (1994). A man skilled in the art knows that such microorganisms are available from depository authorities, e.g. the American Type Culture Collection (ATCC), the Centraalbureau voor  
10 Schimmelcultures (CBS) or the Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH (DSM) or any other depository authority as listed in the Journal "Industrial Property" [(1991) 1, pages 29-40]. Bacteria which can be used are e.g. *E. coli*; Bacilli as, e.g., *Bacillus subtilis*; or  
15 *Streptomyces*, e.g. *Streptomyces lividans* (see e.g. Anné and Mallaert in *FEMS Microbiol. Lett.* 114, 121 (1993)). Preferred *E. coli* strains, which can be used are *E. coli* K12 strains e.g. M15 [described as DZ 291 by Villarejo et al. in *J. Bacteriol.* 120, 466-474 (1974)], HB 101 [ATCC No. 33694] or *E. coli* SG13009  
20 [Gottesman et al., *J. Bacteriol.* 148, 265-273 (1981)].

Vectors which can be used for expression in fungi are known in the art and described e.g. in EP 420358, or by Cullen et al. [*Bio/Technology* 5, 369-376 (1987)], Ward [*Molecular Industrial Mycology, Systems and Applications for Filamentous*  
25 *Fungi*, Marcel Dekker, New York (1991)], Upshall et al. [*Bio/Technology* 5, 1301-1304 (1987)], Gwynne et al. [*Bio/Technology* 5, 71-79 (1987)], or Punt et al. [*J. Biotechnol.* 17, 19-34 (1991)]; and for yeasts by Sreekrishna et al. [*J. Basic Microbiol.* 28, 265-278 (1988), *Biochemistry* 28, 4117-4125  
30 (1989)], Hitzemann et al. [*Nature* 293, 717-722 (1981)] or in EP 183070, EP 183071, EP 248227, or EP 263311. Suitable vectors

which can be used for expression in *E. coli* are mentioned, e.g. by Sambrook et al. [s.a.], Fiers et al. [Procd. 8th Int. Biotechnology Symposium", Soc. Franc. de Microbiol., Paris (Durand et al., eds.), pp. 680-697 (1988)], Bujard et al. [Meth. 5 Enzymol. 155, 416-433 (1987)], or Stüber et al. [Immunological Methods, eds. Lefkovits and Pernis, Academic Press, Inc., Vol. IV, 121-152 (1990)]. Vectors that can be used for expression in Bacilli are known in the art and described, e.g. in EP 207459, EP 405370, Proc. Natl. Acad. Sci. USA 81, 439 (1984) or Yansura 10 and Henner, Meth. Enzymol. 185, 199-228 (1990). Vectors which can be used for the expression in *H. Polymorpha* are known in the art and described, e.g. in Gellissen et al., Biotechnology 9, 291-295 (1991).

Either such vectors already carry regulatory elements, 15 e.g. promoters, or the DNA sequences of the present invention can be engineered to contain such elements. Suitable promotor elements which can be used are known in the art and are, e.g. for *Trichoderma reesei* the *cbh1*- [Haarki et al., Biotechnology 7, 596-600 (1989)] or the *pk11*-promotor [Schindler et al., Gene 20 130, 271-275 (1993)]; for *Aspergillus oryzae* the *amy*-promotor [Christensen et al., Abstr. 19th Lunteren Lectures on Molecular Genetics F23 (1987), Christensen et al., Biotechnology 6, 1419-1422 (1988), Tada et al., Mol. Gen. Genet. 229, 301 (1991)]; and for *Aspergillus niger* the *glaA*- [Cullen et al., Bio/Technology 25 5, 369-376 (1987), Gwynne et al., Bio/Technology 5, 713-719 (1987), Ward in Molecular Industrial Mycology, Systems and Applications for Filamentous Fungi, Marcel Dekker, New York, 83-106 (1991)], *alcA*- [Gwynne et al., Bio/Technology 5, 718-719 (1987)], *suc1*- [Boddy et al., Curr. Genet. 24, 60-66 (1993)], 30 *aphA*- [MacRae et al., Gene 71, 339-348 (1988), MacRae et al., Gene 132, 193-198 (1993)], *tpiA*- [McKnight et al., Cell 46, 143-

147 (1986), Upshall et al., Bio/Technology 5, 1301-1304 (1987)],  
gpdA- [Punt et al., Gene 69, 49-57 (1988), Punt et al., J.  
Biotechnol. 17, 19-37 (1991)] and the pkiA-promotor [de Graaff  
et al., Curr. Genet. 22, 21-27 (1992)]. Suitable promotor  
5 elements that can be used for expression in yeast are known in  
the art and are, e.g. the pho5-promotor [Vogel et al., Mol.  
Cell. Biol., 2050-2057 (1989); Rudolf and Hinnen, Proc. Natl.  
Acad. Sci. 84, 1340-1344 (1987)] or the gap-promotor for  
expression in *Saccharomyces cerevisiae*; the aox1-promotor [Koutz  
10 et al., Yeast 5, 167-177 (1989); Sreekrishna et al., J. Basic  
Microbiol. 28, 265-278 (1988)] for *Pichia pastoris*; or the FMD  
promoter [Hollenberg et al., EPA No. 0299108] or MOX-promotor  
[Ledeboer et al., Nucl. Acids Res. 13, 3063-3082 (1985)] for *H.*  
*polymorpha*.

15 Accordingly vectors comprising DNA sequences of the  
present invention, preferably for the expression of said DNA  
sequences in bacteria or a fungal or a yeast host and such  
transformed bacteria or fungal or yeast hosts are also a part of  
the invention.

20 The invention also provides a system that allows for high  
expression of proteins, in particular of the phytases of the  
invention, such as recombinant *Hansenula* strains. To achieve  
that, the codons of the DNA sequence of such a protein may be  
selected on the basis of a codon frequency table of the organism  
25 used for expression, e.g. of yeast as in the present case (see  
e.g. in Example 1). Optionally, the codons for the signal  
sequence may be selected in a manner as described for the  
specific case in Example 1; that means that a codon frequency  
table is prepared on the basis of the codons used in the DNA  
30 sequences which encode the amino acid sequences of the given  
protein family. Then the codons for the design of the DNA

sequence of the signal sequence are selected from a codon frequency table of the host cell used for expression whereby always codons of comparable frequency in both tables are used.

Once such DNA sequences have been expressed in an appropriate host cell in a suitable medium, the encoded protein can be isolated either from the medium in the case the protein is secreted into the medium or from the host organism in case such protein is present intracellularly by methods known in the art of protein purification or described in case of a phytase, e.g. in EP 420358. Accordingly, a process for the preparation of a polypeptide of the present invention wherein transformed bacteria or a host cell as described above are cultured under suitable culture conditions, and the polypeptide is recovered therefrom and a polypeptide when produced by such a process; or a polypeptide encoded by a DNA sequence of the present invention, are also a part of the present invention.

Once obtained, the polypeptides of the present invention can be characterized regarding their properties that make them useful in agriculture by any assay known in the art.

In general, the polypeptides of the present invention can be used without being limited to a specific field of application, e.g. in case of phytases for the conversion of inositol polyphosphates, like phytate, to inositol and inorganic phosphate.

Furthermore, the polypeptides of the present invention can be used in a process for the preparation of a pharmaceutical composition or compound food or feeds wherein the components of such a composition are mixed with at least one polypeptide of the present invention. Accordingly, compound food or feeds or pharmaceutical compositions comprising at least one polypeptide of the present invention are also a part of the present

invention. A man skilled in the art is familiar with their process of preparation. Such pharmaceutical compositions or compound foods or feeds can further comprise additives or components generally used for such purpose and known in the  
5 state of the art.

The present invention also provides a process for the reduction of levels of phytate in animal manure wherein an animal is fed such a feed composition in an amount effective in converting phytate contained in the feedstuff to lower inositol  
10 phosphates and/or inositol, and inorganic phosphate.

In the present context, a phytase is an enzyme or polypeptide that has phytase activity. A phytase can be e.g. a myo-inositol hexakisphosphate phosphohydrolase, such as (myo-inositol hexakisphosphate 3-phosphohydrolase, EC 3.1.3.8) and  
15 (myo-inositol hexakisphosphate 6-phosphohydrolase, EC 3.1.3.26).

In one embodiment, the phytase is purified, viz. at least 85%, preferably at least 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99% pure, as evaluated by SDS-PAGE. The phytase may be isolated. Phytase activity can be determined using any  
20 phytase assay known in the art, e.g. the assay described herein (see Example 9). The assay temperature may be the optimum temperature of the actual phytase, and the assay pH may be the optimum pH of the actual phytase.

The assay temperature may e.g. be selected within the  
25 range of 20-90°C, or 30-80°C, or 35-75°C, for instance temperatures of 37°C, 50°C, 60°C, or 70°C.

The assay pH may e.g. be selected within the range of pH 2-9, or 3-8, or 3-6, for instance assay pH values of 3, 4, 5, 6, or 7 may be chosen.

30 Amino acid sequence homology (or polypeptide or amino acid homology) is determined as the degree of identity between two

sequences. This may suitably be determined by means of computer programs known in the art such as GAP provided in the GCG program package [Program Manual for the Wisconsin Package, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin 53711, USA], see also Needleman, S.B. and Wunsch, C.D., (1970), J. Mol. Biol., 48, 443-453]. In release 9.1, for comparing polypeptide sequences, the Length Weight is set to 0, and the Gap Weight is set to 3.0.

The degree of identity or homology between two DNA (nucleic acid) sequences may be determined by means of computer programs known in the art such as GAP provided in the GCG program package [Program Manual for the Wisconsin Package, Genetics Computer Group, 575 Science Drive, Madison, Wisconsin 53711, USA], see also Needleman, S.B. and Wunsch, C.D., (1970), J. Mol. Biol., 48, 443-453]. In release 9.1, GAP is used with the following settings for DNA sequence comparison: GAP creation penalty of 50 and GAP extension penalty of 3.

Suitable experimental conditions for determining whether a given DNA or RNA sequence hybridizes to a specified nucleotide or oligonucleotide probe involves presoaking of the filter containing the DNA or RNA fragments to examine for hybridization in 5 x SSC (Sodium chloride/Sodium citrate; (J. Sambrook, E.F. Fritsch, and T. Maniatis, 1989, Molecular Cloning, A Laboratory Manual, 2nd edition, Cold Spring Harbor, New York) for 10 min, and prehybridization of the filter in a solution of 5 x SSC, 5 x Denhardt's solution, 0.5 % SDS and 100 µg/ml of denatured sonicated salmon sperm DNA (Sambrook et al. 1989), followed by hybridization in the same solution containing a concentration of 10 ng/ml of a random-primed (Feinberg, A. P. and Vogelstein, B. (1983) Anal. Biochem. 132:6-13), <sup>32</sup>P-dCTP-labeled (specific

activity >  $1 \times 10^9$  cpm/ $\mu$ g) probe for 12 hours at approximately 45°C.

The filter is then washed twice for 30 minutes in 2 x SSC, 0.5 % SDS at at least 55°C (low stringency), at at least 60°C (medium stringency), at at least 65°C (medium/high stringency), at at least 70°C (high stringency), or at at least 75°C (very high stringency).

Molecules to which the oligonucleotide probe hybridizes under these conditions can be detected using an x-ray film.

10       Phytases of amended thermostability, or thermostable phytases, are one aspect of the present invention. A "thermostable" phytase is a phytase that has a  $T_m$  (melting temperature) - as measured on purified phytase protein by Differential Scanning Calorimetry (DSC) - of at least 65°C. For 15 the DSC, a constant heating rate may be used, e.g. of 10°C/min. In alternative embodiments, the  $T_m$  is at least 66, 67, 68, 69, 70, 71, 72, 73, 74 or 75°C. Or, the  $T_m$  is equal to or lower than 150°C, or equal to or lower than 145, 140, 135, 130, 125, 120, 115 or 110°C. Accordingly, examples of intervals of  $T_m$  are: 65- 20 150°C, 66-150°C, - (etc.) - 75-150°C; 65-145°C, 66-145°C, - (etc.) - 75-145°C; 65-140°C, - (etc.) - 75-140°C; - (etc.) - 65-110°C, 66-110°C, - (etc.) - 75-110°C.

Particular ranges for  $T_m$  are the following: between 65 and 110°C; between 70 and 110°C; between 70 and 100°C; between 75 25 and 95°C, or between 80 and 90°C.

In Examples 9 and 10 below, the measurement of  $T_m$  by DSC is described, and the  $T_m$ 's of a number of phytases are shown.

The optimum temperatures are also indicated, since - as an alternative mean - a thermostable phytase can be defined as a 30 phytase having a temperature-optimum of at least 60°C. Preferably, the optimum temperature is determined on the

substrate phytate or phytic acid at pH 5.0 or 5.5. Example 9 describes an example of a phytase assay, including a definition of units.

In alternative embodiments, the optimum temperature is at least 61, 62, 63, 64, 65, 66, 67, 68, 69 or 70°C. In a particular embodiment, the optimum temperature is equal to or lower than 140°C, or equal to or lower than 135, 130, 125, 120, 115, 110, 105 or 100°C. Accordingly, examples of intervals of optimum temperature are: 60-140°C, 61-140°C, - (etc.) - 70-140°C; 60-135°C, 61-135°C, - (etc.) - 70-135°C; 60-130°C, - (etc.) - 70-130°C; - (etc.) - 60-100°C, 61-100°C, - (etc.) - 70-100°C.

Before describing the present invention in more detail a short explanation of the Figures enclosed is given below.

15

Figure 1: Design of the consensus phytase-1 sequence. The letters represent the amino acid residues in the one-letter code. The following sequences were used for the alignment: phyA from *Aspergillus terreus* 9A-1 [Mitchell, D. B., Vogel, K., Weimann, B. J., Pasamontes, L. & van Loon, A. P. G. M. (1997) The phytase subfamily of histidine acid phosphatases: isolation of genes for two novel phytases from the fungi *Aspergillus terreus* and *Myceliophthora thermophila*, *Microbiology* 143, 245-252); from amino acid (aa) 27; SEQ ID NO: 1]; phyA from *A. terreus* cbs116.46 [EP 897985]. A heat resistant phytase of *Aspergillus fumigatus* with superior performance in animal experiments. Phytase optimization and natural variability. In: *The Biochemistry of phytate and phytases* (eds. Rasmussen, S.K; Raboy, V.; Dalbøge, H. and Loewus, F.; Kluwer Academic Publishers); from aa 27; SEQ ID NO: 2; phyA from *Aspergillus niger* var. *awamori* (Piddington et al (1993) *Gene* 133, 55-62;

from aa 27; SEQ ID NO: 3); phyA from *A. niger* T213 (EP 897985);  
from aa 27; SEQ ID NO: 4); phyA from *A. niger* strain NRRL3135  
[van Hartingsveldt, W., van Zeijl, C. M. F., Hartevelde, G. M.,  
Gouka, R. J., Suykerbuyk, M. E. G., Luiten, R. G. M., van  
5 Paridon, P. A., Selten, G. C. M., Veenstra, A. E., van Gorcom,  
R. F. M., & van den Hondel, C. A. M. J. J. (1993) Cloning,  
characterization and overexpression of the phytase-encoding gene  
(phyA) of *Aspergillus niger*. *Gene* 127, 87-94; from aa 27; SEQ ID  
NO: 5]; phyA from *Aspergillus fumigatus* ATCC 13073 (Pasamontes,  
10 L., Haiker, M., Wyss, M., Tessier, M. & van Loon, A. P. G. M.  
(1997) Cloning, purification and characterization of a heat  
stable phytase from the fungus *Aspergillus fumigatus*, *Appl.*  
*Environ. Microbiol.* 63, 1696-1700; from aa 25; SEQ ID NO: 6];  
phyA from *A. fumigatus* ATCC 32722 (EP 897985); from aa 27; SEQ  
15 ID NO: 7); phyA from *A. fumigatus* ATCC 58128 (EP 897985); from  
aa 27; SEQ ID NO: 8); phyA from *A. fumigatus* ATCC 26906  
(EP 897985); from aa 27; SEQ ID NO: 9); phyA from *A. fumigatus*  
ATCC 32239 (EP 897985); from aa 30; SEQ ID NO: 10; phyA from  
*Emericella nidulans* [Pasamontes, L., Haiker, M., Henriquez-  
20 Huecas, M., Mitchell, D. B. & van Loon, A. P. G. M. (1997a).  
Cloning of the phytases from *Emericella nidulans* and the  
thermophilic fungus *Talaromyces thermophilus*. *Biochim. Biophys.*  
*Acta* 1353, 217-223; from aa 25; SEQ ID NO: 11]; phyA from  
*Talaromyces thermophilus* (Pasamontes et al., 1997a; from aa 24;  
25 SEQ ID NO: 12); and phyA from *Myceliophthora thermophila*  
(Mitchell et al., 1997; from aa 19; SEQ ID NO: 13). The  
alignment was calculated using the program PILEUP. The location  
of the gaps was refined by hand. Capitalized amino acid residues  
in the alignment at a given position belong to the amino acid  
30 coalition that establish the consensus residue. In bold, beneath  
the calculated consensus sequence, the amino acid sequence of

the finally constructed consensus phytase (Fcp) is shown (SEQ ID NO: 14). The gaps in the calculated consensus sequence were filled by hand according to principals stated in Example 1.

5        Figure 2: DNA sequence (SEQ ID NO: 15) of the consensus phytase-1 gene (fcp) and of the primers used for the gene construction. The calculated amino acid sequence (Figure 1, SEQ ID NO: 14) was converted into a DNA sequence using the program BACKTRANSLATE [Devereux, J., Haeberli, P. & Smithies, O. (1984)  
10 A comprehensive set of sequence analysis programs for the VAX. Nucl. Acids Res. 12, 387-395], and the codon frequency table of highly expressed yeast genes (GCG program package, 9.0). The signal peptide of the phytase from *A. terreus* cbs 116.46 was fused to the N-terminus. The amino acid sequence shown in Fig. 2  
15 is SEQ ID NO: 16. The bold bases represent the sequences of the oligonucleotides used to generate the gene. The names of the respective oligonucleotides are alternately noted above or below the sequence. The underlined bases represent the start and stop codon of the gene. The bases written in italics represent the  
20 two introduced Eco RI sites.

Figure 3: Alignment and consensus sequence of five Basidiomycete phytases. The letters represent the amino acid residues in the one-letter code. The amino acid sequences of the  
25 phytases from *Paxillus involutus*, phyA1 (from aa 21; SEQ ID NO: 17; and phyA2 (from aa 21, WO 98/28409; SEQ ID NO: 18); *Trametes pubescens* (from aa 24, WO 98/28409; SEQ ID NO: 19); *Agrocybe pediades* (from aa 19, WO 98/28409; SEQ ID NO: 20); and *Peniophora lycii* (from aa 21, WO 98/28409; SEQ ID NO: 21),  
30 starting with the amino acid residues mentioned in parentheses, were used for the alignment and the calculation of the

corresponding consensus sequence called "Basidio" (Example 2; SEQ ID NO: 22). The alignment was performed with the program PILEPUP. The location of the gaps was refined by hand. The consensus sequence was calculated by the program PRETTY. While a  
5 vote weight of 0.5 was assigned to the two *P. involutus* phytases, all other genes were used with a vote weight of 1.0 for the consensus sequence calculation. At positions where the program was not able to determine a consensus residue, the Basidio sequence contains a dash. Capitalized amino acid  
10 residues in the alignment at a given position represent the amino acid coalition that established the consensus residue.

Figure 4: Design of consensus phytase-10 amino acid sequence. By adding the sequence of *Thermomyces lanuginosus*  
15 phytase [Berka, R. M., Rey, M. W., Brown, K. M., Byun, T. & Klotz, A. V. (1998) Molecular characterization and expression of a phytase gene from the thermophilic fungus *Thermomyces lanuginosus*. Appl. Environ. Microbiol. 64, 4423-4427; SEQ ID NO: 23] and the consensus sequence of the phytases from five  
20 Basidiomycetes (SEQ ID NO: 22) to the alignment of Figure 1, an improved consensus sequence was calculated by the program PRETTY. Additionally, the amino acid sequence of *A. niger* T213 was omitted, and a vote weight of 0.5 was assigned to the remaining two *A. niger* phytase sequences. For further  
25 information see Example 2.

Figure 5: DNA and amino acid sequence of consensus phytase-10 (SEQ ID NO: 25, and SEQ ID NO: 26, respectively). The amino acid sequence is written above the corresponding DNA  
30 sequence using the one-letter code. The sequence of the oligonucleotides that were used to assemble the gene are in

bold letters. The names of the respective oligonucleotides and the amino acids that differ relative to consensus phytase-1 are underlined. The fcp10 gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6, 5 CP-7.10, CP-8.10, CP-9.10, CP-10.10, CP-11.10, CP-12.10, CP-13.10, CP-14.10, CP-15.10, CP-16.10, CP-17.10, CP18.10, CP-19.10, CP-20.10, CP-21.10, and CP-22.10. The newly synthesized oligonucleotides are additionally marked by the number 10. The phytase contains the following 32 exchanges relative to 10 consensus phytase-1: Y54F, E58A, D69K, D70G, A94K, N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V, A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V, R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E. The underlined mutations revealed a stabilizing effect on consensus phytase-1 15 when tested as single mutations in consensus phytase-1.

Figure 6: Alignment for the design of consensus phytase-11 (SEQ ID NO: 27). In contrast to the design of consensus phytase-10, for the design of the amino acid sequence of consensus 20 phytase-11, all Basidiomycete phytases were used as independent sequences using an assigned vote weight of 0.2 for each Basidiomycete sequence. Additionally, the amino acid sequence of *A. niger* T213 was again used in this alignment.

25 Figure 7: DNA and amino acid sequence of consensus phytase-1-thermo[8]-Q50T-K91A (SEQ ID NO: 28, and SEQ ID NO: 29, respectively). The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues (relative to consensus phytase-1) 30 are underlined. The stop codon of the gene is marked by a star (\*).

Figure 8: DNA and amino acid sequence of consensus phytase-10-thermo[3]-Q50T-K91A (SEQ ID NO: 30, and SEQ ID NO: 31, respectively). The amino acid sequence is written above the  
5 corresponding DNA sequence using the one-letter code. The replaced amino acid residues (relative to consensus phytase-10) are underlined. The stop codon of the gene is marked by a star (\*).

10 Figure 9: DNA and amino acid sequence of *A. fumigatus* ATCC 13073 phytase alpha-mutant Q51T (SEQ ID NO: 32, and SEQ ID NO: 33, respectively). The amino acid sequence is written above the corresponding DNA sequence using the one-letter code. The replaced amino acid residues (relative to *A. fumigatus* ATCC  
15 13073 phytase) are underlined. The stop codon of the gene is marked by a star (\*).

Figure 10: DNA and amino acid sequence of consensus phytase-7 (SEQ ID NO: 34, and SEQ ID NO: 35, respectively). The  
20 amino acids are written above the corresponding DNA sequence using the one-letter code. The sequence of the oligonucleotides used to assemble the gene are in bold letters. Oligonucleotides and amino acids that were exchanged (relative to consensus phytase-1) are underlined and the corresponding triplets are  
25 written in small case letters. The fcp7 gene was assembled from the following oligonucleotides: CP-1, CP-2, CP-3, CP-4.7, CP-5.7, CP-6, CP-7, CP-8.7, CP-9, CP-10.7, CP-11.7, CP-12.7, CP-13.7, CP-14.7, CP-15.7, CP-16, CP-17.7, CP-18.7, CP-19.7, CP-20, CP-21, and CP-22. The newly synthesized oligonucleotides are  
30 additionally marked by the number 7. Consensus phytase-7 contains the following 24 exchanges in comparison to the

original consensus phytase-1: S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S.

5

Figure 11: Differential scanning calorimetry (DSC) of consensus phytase-1 and consensus phytase-10. The protein samples were concentrated to about 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-10 (upper graph) yielded a melting temperature of 85.4°C, which is 7.3°C higher than the melting point of consensus phytase-1 (78.1°C, lower graph).

15 Figure 12: Differential scanning calorimetry (DSC) of consensus phytase-10-thermo[3]-Q50T and consensus phytase-10-thermo[3]-Q50T-K91A. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-10-thermo[3]-Q50T (upper graph) yielded a melting temperature of 88.6°C, while the melting temperature of consensus phytase-10-thermo-Q50T-K91A was determined to be 89.3°C.

25 Figure 13: Comparison of the temperature optimum between consensus phytase-1, consensus phytase-10 and consensus phytase-10-thermo[3]-Q50T. For the determination of the temperature optimum, the phytase standard assay of Example 9 was performed at a series of temperatures between 37 and 86°C. The diluted supernatant of transformed *S. cerevisiae* strains was used for the determination. The other components of the supernatant had

30

no influence on the determination of the temperature optimum:  $\wedge$ , consensus phytase-1;  $\diamond$ , consensus phytase-10;  $\blacksquare$ , consensus phytase 10-thermo[3]-Q50T.

5        Figure 14: pH-dependent activity profile and substrate specificity of consensus phytase-10 and its variants thermo[3]-Q50T and thermo[3]-Q50T-K91A. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows the pH-  
10 dependent activity profile of consensus phytase-10 ( $\square$ ), consensus phytase-10-thermo[3]-Q50T ( $\bullet$ ), and consensus phytase-10-thermo[3]-Q50T-K91A ( $\wedge$ ). Graph b) shows the corresponding substrate specificity tested by replacement of phytate in the standard assay by the indicated compounds; open bars, consensus  
15 phytase-10; grey bars, consensus phytase-10-thermo[3]-Q50T; dark bars, consensus phytase-10-thermo[3]-Q50T-K91A). The numbers correspond to the following substrates: 1, phytate; 2, p-nitrophenyl phosphate; 3, phenyl phosphate; 4, fructose-1,6-bisphosphate; 5, fructose-6-phosphate; 6, glucose-6-phosphate;  
20 7, ribose-5-phosphate; 8, DL-glycerol-3-phosphate; 9, glycerol-2-phosphate; 10, 3-phosphoglycerate; 11, phosphoenolpyruvate; 12, AMP; 13, ADP; 14, ATP.

Figure 15: pH-dependent activity profile and substrate  
25 specificity of consensus phytase-1-thermo[8]-Q50T and of consensus phytase-1-thermo[8]-Q50T-K91A. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows the pH-dependent activity profile of the Q50T- ( $\blacksquare$ ) and the Q50T-K91A-

variant (.). Graph b) shows the corresponding substrate specificities tested by replacement of phytate in the standard assay by the indicated compounds (open bars, consensus phytase-1-thermo[8]-Q50T; filled bars, consensus phytase-1-thermo[8]-Q50T-K91A). The substrates are listed in the legend of Figure 14.

Figure 16: Differential scanning calorimetry (DSC) of consensus phytase-1-thermo[8]-Q50T and consensus phytase-1-thermo[8]-Q50T-K91A. The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of consensus phytase-1-thermo[8]-Q50T (upper graph) showed a melting temperature of 84.7°C, while the melting point of consensus phytase-1-thermo[8]-Q50T-K91A was found at 85.7°C.

Figure 17: Comparison of the temperature optimum between consensus phytase-1, consensus phytase-1-thermo[3] and consensus phytase-1-thermo[8]. For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 37 and 86°C. Protein purified from the supernatant of transformed *S. cerevisiae* strains was used for the determination. ○, consensus phytase-1; □, consensus phytase-1-thermo[3]; ▲, consensus phytase 1-thermo[8].

Figure 18: Comparison of the pH-dependent activity profile and substrate specificity between consensus phytase-1, consensus phytase-7, and the phytase from *A. niger* NRRL 3135. The phytase activity was determined using the standard assay in appropriate buffers (see Example 9) at different pH-values. Graph a) shows

the pH-dependent activity profile of consensus phytase-1 (■), the phytase from *A. niger* NRRL 3135 (○), and of consensus phytase-7 (▲). Graph b) shows the corresponding substrate specificities tested by replacement of phytate in the standard assay by the indicated compounds (black bars, *A. niger* NRRL 3135 phytase; open bars, consensus phytase-1; dashed bars, consensus phytase-7). The substrates are listed in the legend of Figure 14.

10      Figure 19: Differential scanning calorimetry (DSC) of the phytase from *A. fumigatus* ATCC 13073 and of its stabilized alpha-mutant, which contains the following amino acid exchanges: F55Y, V100I, F114Y, A243L, S265P, and N294D.

15      The protein samples were concentrated to ca. 50-60 mg/ml and extensively dialyzed against 10 mM sodium acetate, pH 5.0. A constant heating rate of 10°C/min was applied up to 95°C. DSC of *A. fumigatus* 13073 phytase (lower graph) revealed a melting temperature of 62.5°C, while the melting point of the alpha-mutant was found at 67.0°C.

20

Figure 20: Comparison of the temperature optima of *A. fumigatus* 13073 wild-type phytase, its alpha-mutant, and a further stabilized alpha-mutant (E59A-S154N-R329H-S364T-G404A). For the determination of the temperature optimum, the phytase standard assay was performed at a series of temperatures between 25 37 and 75°C. The diluted supernatant of transformed *S. cerevisiae* strains was used for the determination. The other components of the supernatant had no influence on the determination of the temperature optimum. ○, *A. fumigatus* ATCC 30 13073 phytase; ▲, *A. fumigatus* ATCC 13073 alpha-mutant; □, *A.*

fumigatus ATCC 13073 alpha-mutant-(E59A-S154N-R329H-S364T-G404A)-Q27T; ■, A. fumigatus ATCC 13073 alpha-mutant-(E59A-S154N-R329H-S364T-G404A)-Q51T-K92A. Q51T and K92A correspond to consensus phytase-1 substitutions Q50T and K91A, respectively.

5

Figure 21: Amino acid sequence of consensus phytase-12 (consphy12; SEQ ID NO: 36) which contains a number of active site residues transferred from the "basidio" consensus sequence to consensus phytase-10-thermo[3]-Q50T-K91A (underlined).

10

Figure 22: DNA and amino acid sequence of consensus phytase-3-thermo[11]-Q50T. The amino acids are written below the corresponding DNA sequence using the one-letter code.

15

Figure 23: DNA and amino acid sequence of consensus phytase-3-thermo[11]-Q50T-K91A. The amino acids are written below the corresponding DNA sequence using the one-letter code.

Figure 24: DNA and amino acid sequence of consensus phytase-10-thermo[5]-Q50T. The amino acids are written below the corresponding DNA sequence using the one-letter code.

Figure 25: DNA and amino acid sequence of consensus phytase-10-thermo[5]-Q50T-K91A. The amino acids are written below the corresponding DNA sequence using the one-letter code.

The phytase-producing microorganism strains mentioned herein, viz. Paxillus involutus CBS 100231; Peniophora lycii CBS 686.96; Agrocybe pediades CBS 900.96; and Trametes pubescens CBS 100232; were isolated from natural samples originating from, respectively, Denmark; Denmark; Denmark; and Sweden (the Uppsala

30

collection. The samples were collected in November 1992; October 1993; June 1995; and in November 1995, respectively.

#### Example 1

##### 5 Consensus phytase-1

The amino acid sequence of consensus phytase-1 (fungal consensus phytase, fcp) was designed and calculated as described in Examples 1 and 2 of EP 897985. Table 1 below shows the origin and vote weight of the phytase amino acid sequences used for the  
10 design of consensus phytase-1. The consensus phytase-1 sequence was furthermore converted into a DNA sequence as described in Example 3 of EP 897985, and the consensus phytase-1 gene was constructed and cloned as described in Example 4 of EP 897985.

##### 15 Table 1

###### Origin and vote weight of the phytase amino acid sequences

- phyA from *Aspergillus terreus* 9A-1, aa 27, vote weight 0.5 (Mitchell et al., 1997)
- phyA from *Aspergillus terreus* cbs116.46, aa 27, vote weight  
20 0.5 (EP 897985)
- phyA from *Aspergillus niger* var. *awamori*, aa 27, vote weight 0.33 [Piddington, C. S., Houston, C. S., Paloheimo, M., Cantrell, M., Miettinen-Oinonen, A., Nevalainen, H., & Rambosek, J. (1993) The cloning and sequencing of the genes encoding  
25 phytase (phy) and pH 2.5-optimum acid phosphatase (aph) from *Aspergillus niger* var. *awamori*. Gene 133, 55-62].
- phyA from *Aspergillus niger* T213 (EP 897985), aa 27, vote weight 0.33
- 30 - phyA from *Aspergillus niger* strain NRRL3135, aa 27, vote weight 0.33 (van Hartingsveldt et al., 1993)

- phyA from *Aspergillus fumigatus* ATCC 13073, aa 26, vote weight 0.2 (Pasamontes et al., 1997)
- phyA from *Aspergillus fumigatus* ATCC 32722, aa 26, vote weight 0.2 (EP 897985)
- 5 - phyA from *Aspergillus fumigatus* ATCC 58128, aa 26, vote weight 0.2 (EP 897985)
- phyA from *Aspergillus fumigatus* ATCC 26906, aa 26, vote weight 0.2 (EP 897985)
- phyA from *Aspergillus fumigatus* ATCC 32239, aa 30, vote weight  
10 0.2 (EP 897985)
- phyA from *Emmericella nidulans* , aa 25, vote weight 1.0 (Pasamontes et al., 1997a)
- phyA from *Talaromyces thermophilus* ATCC 20186, aa 24, vote weight 1.0 (Pasamontes et al., 1997a)
- 15 - phyA from *Myceliophthora thermophila*, aa 19, vote weight 1.0 (Mitchell et al., 1997)

### Example 2

#### Design of an improved consensus phytase (consensus 20 phytase-10) amino acid sequence

The alignments used for the design of consensus phytase-10 were calculated using the program PILEUP from the GCG Sequence Analysis Package Release 9.0 (Devereux et al., 1984) with the  
25 standard parameters (gap creation penalty 12, gap extension penalty 4). The location of the gaps was refined using a text editor.

The following sequences were used for the alignment of the Basidiomycete phytases starting with the amino acid (aa)  
30 mentioned in Table 2:

Table 2

Origin and vote weight of five Basidiomycete phytases used for the calculation of the corresponding consensus amino acid sequence (basidio)

5

- phyA1 from Paxillus involutus CBS No. 100231, aa 21, vote weight 0.5 (WO 98/28409)
- phyA2 from Paxillus involutus CBS No. 100231, aa 21, vote weight 0.5 (WO 98/28409)
- 10 - phyA from Trametes pubescens CBS No. 100232, aa 24, vote weight 1.0 (WO 98/28409)
- phyA from Agrocybe pediades CBS No. 900.96, aa 19, vote weight 1.0 (WO 98/28409)
- phyA from Peniophora lycii CBS No. 686.96, aa 21, vote weight 15 1.0 (WO 98/28409)

The alignment is shown in Figure 3.

In Table 3 the genes that were used for the final  
20 alignment are listed. The first amino acid (aa) of the sequence that is used in the alignment is mentioned behind the organism's designation.

Table 3

25 Origin and vote weight of the phytase sequences used for the design of consensus phytase-10

- phyA from Aspergillus terreus 9A-1, aa 27, vote weight 0.5 (Mitchell et al., 1997)
- 30 - phyA from Aspergillus terreus cbs116.46, aa 27, vote weight 0.5 (EP 897985)

- phyA from *Aspergillus niger* var. *awamori*, aa 27, vote weight 0.5 (Piddington et al., 1993)
- phyA from *Aspergillus niger* strain NRRL3135, aa 27, vote weight 0.5 (van Hartingsveldt et al., 1993)
- 5 - phyA from *Aspergillus fumigatus* ATCC 13073, aa 26, vote weight 0.2 (Pasamontes et al., 1997)
- phyA from *Aspergillus fumigatus* ATCC 32722, aa 26, vote weight 0.2 (EP 897985)
- phyA from *Aspergillus fumigatus* ATCC 58128, aa 26, vote weight 10 0.2 (EP 897985)
- phyA from *Aspergillus fumigatus* ATCC 26906, aa 26, vote weight 0.2 (EP 897985)
- phyA from *Aspergillus fumigatus* ATCC 32239, aa 30, vote weight 0.2 (EP 897985)
- 15 - phyA from *Emmericella nidulans* , aa 25, vote weight 1.0 (Pasamontes et al., 1997a)
- phyA from *Talaromyces thermophilus* ATCC 20186, aa 24, vote weight 1.0 (Pasamontes et al., 1997a)
- phyA from *Myceliophthora thermophila*, aa 19, vote weight 1.0 20 (Mitchell et al., 1997)
- phyA from *Thermomyces lanuginosus*, aa 36, vote weight 1.0 (Berka et al., 1998)
- Consensus sequence of five Basidiomycete phytases, vote weight 1.0 (Basidio, Figure 3)

25

The corresponding alignment is shown in Figure 4.

#### Calculation of the amino acid sequence of consensus phytase-10

30 To improve the alignment, we added the original consensus sequence of five phytases from four different Basidiomycetes

(called Basidio; still containing the undefined sequence positions; see Figure 3), nearly all phytase sequences used for the calculation of the original consensus phytase sequences and one new phytase sequence from the Ascomycete *Thermomyces* lanuginosus to a larger alignment.

We set plurality on 2.0 and threshold on 3. The used vote weights are listed in Table 3. The alignment and the corresponding consensus sequence are presented in Figure 4. The new consensus phytase sequence has 32 different amino acids in comparison to the original consensus phytase-1. Positions for which the program PRETTY was not able to calculate a consensus amino acid residue were filled according to rules mentioned in Example 1. None of the residues suggested by the program was replaced.

Furthermore, in another calculation, we included all Basidiomycete phytases as single amino acid sequences but assigning a vote weight of 0.2 in the calculation. The corresponding alignment is shown in Figure 6. The calculated consensus amino acid sequence (consensus phytase-11) has the following differences to the sequence of consensus phytase-10. Letter X means that the program was not able to calculate a consensus amino acid; the amino acid in parenthesis corresponds to the amino acid finally included into consensus phytase-10.

D35X (first letter for consensus phytase-10, last letter for consensus phytase-11), X(K)69K, X(E)100E, A101R, Q134N, X(K)153N, X(H)190H, X(A)204S, X(E)220D, E222T, V227A, X(R)271R, H287A, X(D)288D, X(K)379K, X(I)389I, E390X, X(E)415E, X(A)416A, X(R)446L, E463A. The numbering is as in Fig. 5.

We also checked single amino acid replacements suggested by the improved consensus sequences 10 and 11 on their influence

on the stability of the original consensus phytase-1. The approach is described in example 3.

Conversion of the consensus phytase-10 amino acid sequence  
5 into a DNA sequence

The first 26 amino acid residues of *A. terreus* cbs116.46 phytase were used as signal peptide and fused to the N-terminus of consensus phytase-10. The used procedure is further described in Example 1.

10 The resulting sequence of the fcp10 gene is shown in Figure 5.

Construction and cloning of the consensus phytase-10 gene  
(fcp10)

15 The calculated DNA sequence of fcp10 was divided into oligonucleotides of 85 bp, alternately using the sequence of the sense and the anti-sense strand. Every oligonucleotide overlaps 20 bp with the previous and the following oligonucleotide of the opposite strand. The location of all primers, purchased from  
20 Microsynth, Balgach (Switzerland) and obtained in a PAGE-purified form, is indicated in Figure 5.

PCR-Reactions

In three PCR reactions, the synthesized oligonucleotides  
25 were composed to the entire gene. For the PCR, the High Fidelity Kit from Boehringer Mannheim (Boehringer Mannheim, Mannheim, Germany) and the thermo cycler "The ProtokolTM" from AMS Biotechnology (Europe) Ltd. (Lugano, Switzerland) were used. The following oligonucleotides were used in a concentration of 0.2  
30 pMol/ml.

Mix 1.10: CP-1, CP-2, CP-3.10, CP-4.10, CP-5.10, CP-6,  
CP-7.10, CP-8.10, CP-9.10, CP-10.10

5 Mix 2.10: CP-9.10, CP-11.10, CP-12.10, CP-13.10, CP-  
14.10, CP-15.10, CP-16.10, CP-17.10, CP18.10,  
CP-19.10, CP-20.10, CP-21.10, CP-22.10

The newly synthesized oligonucleotides are marked by the  
number 10. Consensus phytase-10 contains the following 32  
exchanges, which are underlined in Figure 5, in comparison to  
10 the original consensus phytase-1: Y54F, E58A, D69K, D70G, A94K,  
N134Q, I158V, S187A, Q188N, D197N, S204A, T214L, D220E, L234V,  
A238P, D246H, T251N, Y259N, E267D, E277Q, A283D, R291I, A320V,  
R329H, S364T, I366V, A379K, S396A, G404A, Q415E, A437G, A463E.

Four short PCR primers were used for the assembling of the  
15 oligonucleotides:

CP-a: *Eco RI*

5'-TATATGAATTCATGGGCGTGTTTCGTC-3' (SEQ ID NO: 37)

20 CP-b:

5'-TGAAAAGTTCATTGAAGGTTTC-3' (SEQ ID NO: 38)

CP-c.10:

5'-TCTTCGAAAGCAGTACACAAAC-3' (SEQ ID NO: 39)

25

CP-e: *Eco RI*

5'-TATATGAATTCTTAAGCGAAAC-3' (SEQ ID NO: 40)

35

PCR reaction a: 10  $\mu$ l Mix 1.10 (2.0 pmol of each  
oligonucleotide)

2  $\mu$ l nucleotides (10 mM of each nucleotide)

2  $\mu$ l primer CP-a (10 pmol/ml)

5 2  $\mu$ l primer CP-c.10 (10 pmol/ml)

10,0  $\mu$ l PCR buffer

0.75  $\mu$ l polymerase mixture (2.6 U)

73.25  $\mu$ l H<sub>2</sub>O

10 PCR reaction b: 10  $\mu$ l Mix 2.10 (2.0 pmol of each  
oligonucleotide)

2  $\mu$ l nucleotides (10 mM each nucleotide)

2  $\mu$ l primer CP-b (10 pmol/ml)

2  $\mu$ l primer CP-e (10 pmol/ml)

15 10,0  $\mu$ l PCR buffer

0.75  $\mu$ l polymerase mixture (2.6 U)

73.25  $\mu$ l H<sub>2</sub>O

Reaction conditions for PCR reactions a and b:

20            step 1            2 min - 45°C  
             step 2            30 sec - 72°C  
             step 3            30 sec - 94°C  
             step 4            30 sec - 52°C  
             step 5            1 min - 72°C

25

Steps 3 to 5 were repeated 40-times.

The PCR products (670 and 905 bp) were purified by agarose  
gel electrophoresis (0.9% agarose), followed by gel extraction  
30 (QIAEX II Gel Extraction Kit, Qiagen, Hilden, Germany). The  
purified DNA fragments were used for the PCR reaction c.

PCR reaction c: 6  $\mu$ l PCR product of reaction a  $\approx$ 50 ng)  
6  $\mu$ l PCR product of reaction b  $\approx$ 50 ng)  
2  $\mu$ l primer CP-a (10 pmol/ml)  
5 2  $\mu$ l primer CP-e (10 pmol/ml)  
10,0  $\mu$ l PCR buffer  
0.75  $\mu$ l polymerase mixture (2.6 U)  
73.25  $\mu$ l H<sub>2</sub>O

10 Reaction conditions for PCR reaction c:

step 1	2 min - 94°C
step 2	30 sec - 94°C
step 3	30 sec - 55°C
step 4	1 min - 72°C

15

Steps 2 to 4 were repeated 31-times.

The resulting PCR product (1.4 kb) was purified as mentioned above, digested with EcoRI, and ligated in an EcoRI-  
20 digested and dephosphorylated pBsk(-)-vector (Stratagene, La Jolla, CA, USA). 1  $\mu$ l of the ligation mixture was used to transform E. coli XL-1 competent cells (Stratagene, La Jolla, CA, USA). All standard procedures were carried out as described by Sambrook et al. (1987). The DNA sequence of the constructed  
25 gene (fcp10) was checked by sequencing as known in the art.

Example 3

Increasing the thermostability of consensus phytase-1 by  
introduction of single mutations suggested by the amino  
acid sequences of consensus phytase-10 and consensus  
phytase-11

In order to increase the thermostability of homologous genes, it is also possible to test the stability effect of each differing amino acid residue between the protein of interest and the calculated consensus sequence and to combine all stabilizing mutations into the protein of interest. We used the consensus phytase-1 as protein of interest and tested the effect on the protein stability of 34 amino acid residues that differ relative to consensus phytase-10 and/or -11 by single site-directed mutagenesis.

To construct muteins for expression in *A. niger*, *S. cerevisiae*, or *H. polymorpha*, the corresponding expression plasmid containing the consensus phytase-1 gene was used as template for site-directed mutagenesis (see Examples 6-8). Mutations were introduced using the "quick exchange™ site-directed mutagenesis kit" from Stratagene (La Jolla, CA, USA) following the manufacturer's protocol and using the corresponding primers. All mutations made and the corresponding primers are summarized in Table 4. Plasmids harboring the desired mutation were identified by DNA sequence analysis as known in the art.

Table 4Primers used for site-directed mutagenesis of consensus phytase-  
1

Exchanged bases are highlighted in bold. The introduction of a  
5 restriction site is marked above the sequence. When a  
restriction site is written in parenthesis, the mentioned site  
was destroyed by introduction of the mutation.

mutation	Primer set
	<i>Kpn</i> I
10 Q50T	5' - CACTTGTGGGG <b>TAC</b> CTACTCTCCATACTTCTC-3' (SEQ ID NO: 41) 5' - GAGAAGTATGGAGAGTAG <b>GT</b> ACCCACAAAGTG-3'
Y54F	5' - GGTC AATACTCTCCATTCTTCTCTTTGGAAG-3' (SEQ ID NO: 42)
15	5' - CTTCCAAAGAGAAGA <b>A</b> TGGAGAGTATTGACC-3'
E58A	5' - CATACTTCTCTTTGGCAGACGAATCTGC-3' (SEQ ID NO: 43) 5' - GCAGATTCGTCTGCCAAAGAGAAGTATG-3'
20	<i>Aat</i> II
D69K	5' - CTCCAGACGT <b>CCCAA</b> AGGACTGTAGAGTTAC-3' (SEQ ID NO: 44) 5' - GTAACCTCTACAGTC <b>CTTT</b> GGGACGTCTGGAG-3'
	<i>Aat</i> II
25 D70G	5' - CTCCAGACGT <b>CC</b> CAGACGGCTGTAGAGTTAC-3' (SEQ ID NO: 45) 5' - GTAACCTCTACAGCCGTCTGG <b>G</b> ACGTCTGGAG-3'
K91A	5' - GATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTG-3' (SEQ ID NO: 46)
30	5' - CAGAGTAAGCCTTAGACGCAGAAGAAGTTGGGTATC-3'
	<i>Sca</i> I
A94K	5' - CTTCTAAGTCTAAGA <b>AG</b> TACTCTGCTTTG-3' (SEQ ID NO: 47) 5' - CAAAGCAGAGTACT <b>TT</b> CTTAGACTTAGAAG-3'
35	
A101R	5' - GCTTACTCTGCTTTGATTGAACGGATTCAAAGAACGCTAC-3' (SEQ ID NO: 48) 5' - GTAGCGTTCTTTTGAAT <b>CC</b> GTTCATCAAAGCAGAGTAAGC-3'
40 N134Q	5' - CCATTCGGTGAACAGCAAATGGTTAACTC-3' (SEQ ID NO: 49) 5' - GAGTTAACCATTGCTGTTCAACCGAATGG-3'

		<i>Nru I</i>	
	K153N	5' -GATACAAGGCTCTCGCGAGAAACATTGTTC -3' (SEQ ID NO: 50)	
		5' -GGAACAATGTTTCTCGCGAGAGCCTTGTATC-3'	
5		<i>Bss HI</i>	
	I158V	5' -GATTGTTCCATTTCGTGCGCGCTTCTGGTTC-3' (SEQ ID NO: 51)	
		5' -GAACCAGAAGCGCGCACGAATGGAACAATC-3'	
		<i>Apa I</i>	
10	S187A	5' -GGCTGACCCAGGGGCCCAACCACACCAAGC-3' (SEQ ID NO: 53)	
		5' -GCTTGGTGTGGTTGGGCCCTGGGTCAGCC-3'	
		<i>Bcl I</i>	
	D197N	5' -CTCCAGTTATTAACGTGATCATTCCAGAAGG-3' (SEQ ID NO: 52)	
15		5' -CCTTCTGGAATGATCACGTTAATAACTGGAG-3'	
		<i>Nco I</i>	
	T214L	5' -CACTTTGGACCATGGTCTTTGTACTGCTTTTCG-3' (SEQ ID NO: 54)	
20		5' -CGAAAGCAGTACAAAGACCA TGGTCCAAAGTG-3'	
		<i>Avr II</i>	
	E222T	5' -GCTTTCGAAGACTCTACCC TAGGTGACGACGTTG-3' (SEQ ID NO: 55)	
		5' -CAACGTCGTCACCTAGGGTAGAGTCTTCGAAAGC-3'	
25		<i>V227A</i>	
	V227A	5' -GGTGACGACGCTGAAGCTAACTTCAC-3' (SEQ ID NO: 56)	
		5' -GTGAAGTTAGCTTCAGCGTCGTCACC-3'	
		<i>Sac II</i>	
30	L234V	5' -CTAACTTCACCGCGGTGTTGCTCCAG-3' (SEQ ID NO: 57)	
		5' -CTGGAGCGAACACCGCGGTGAAGTTAG-3'	
	A238P	5' -GCTTTGTTGCTCCACCTATTAGAGCTAGATTGG-3' (SEQ ID NO: 58)	
35		5' -CCAATCTAGCTCTAATAGGTGGAGCGAACAAAGC-3'	
		<i>Hpa I</i>	
	T251N	5' -GCCAGGTGTTAACTTGACTGACGAAG-3' (SEQ ID NO: 59)	
40		5' -TTCGTCAGTCAAGTTAACACCTGGC-3'	
		<i>Aat II</i>	
	Y259N	5' -GACGAAGACGTCGTAACTTGATGGAC-3' (SEQ ID NO: 60)	
		5' -GTCCATCAAGTTAACGACGTCTTCGTC-3'	
45		<i>Asp I</i>	
	E267D	5' -GTCCATTGACACTGTCGCTAGAACTTC-3' (SEQ ID NO: 61)	
		5' -GAAGTTCTAGCGACAGTGTCGAATGGAC-3'	

E277Q 5' -CTGACGCTACTCAGCTGTCTCCATTC-3' (SEQ ID NO: 62)  
5' -GAATGGAGACAGCTGAGTAGCGTCAG-3'

5 A283D 5' -GTCTCCATTCTGTGATTTGTTCACTCAC-3' (SEQ ID NO: 63)  
5' -GTGAGTGAACAAATCACAGAATGGAGAC-3'

*Ksp I*

10 H287A 5' -GCTTTGTTCA**CCGCGG**ACGAATGGAG-3' (SEQ ID NO: 64)  
5' -CTCCATTCGT**CCGCGG**TGAACAAAGC-3'

*Bam HI*

15 R291I 5' -CACGACGAATGGAT**CCA**TACGACTAC-3' (SEQ ID NO: 65)  
5' -GTAGTCGTATTG**GA**TCCATTCGTCGTG-3'

*Bsi WI*

Q292A 5' -GACGAATGGAGAG**CGT**ACGACTACTTG-3' (SEQ ID NO: 66)  
5' -CAAGTAGTCGT**ACGCT**CTCCATTCGTC-3'

*Hpa I*

20 A320V 5' -GGTGT**TGGTTTCGTTA**ACGAATTGATTGC-3' (SEQ ID NO: 67)  
5' -GCAATCAATTCG**TTA**ACGAACCAACACC-3'

*(Bgl II)*

25 R329H 5' -GCTAGATTGACT**CACTCT**CCAGTTCAAG-3' (SEQ ID NO: 68)  
5' -CTTGA**ACTGGAGAGTG**AGTCAATCTAGC-3'

*Eco RV*

30 S364T 5' -CTCACGACAACACTATGAT**ATCT**ATTTTCTTC-3' (SEQ ID NO: 69)  
5' -GAAGAAAATAGAT**ATC**ATAGTGTTGTCGTGAG-3'

*Nco I*

35 I366V 5' -CGACAACT**CCATGG**TTTCTATTTTCTTCGC-3' (SEQ ID NO: 70)  
5' -GCGAAGAAAATAGAA**CCATG**GAGTTGTCG-3'

*Kpn I*

A379K 5' -GTACAACGGT**ACCAAG**CCATTGTCTAC-3' (SEQ ID NO: 71)  
5' -GTAGACAATGG**CTTGGT**ACCGTTGTAC-3'

40 S396A 5' -CTGACGGTTACGCTGCTTCTTGGAC-3' (SEQ ID NO: 72)  
5' -GTCCAAGAAGCAGCGTAACCGTCAG-3'

G404A 5' -CTGTTCCATTCGCTGCTAGAGCTTAC-3' (SEQ ID NO: 73)  
5' -GTAAGCTCTAGCAGCGAATGGAACAG-3'

45 Q415E 5' -GATGCAATGTGAAGCTGAAAAGGAACC-3' (SEQ ID NO: 74)  
5' -GGTTCCTTTT**CAGCTT**CACATTGCATC-3'

*Sal I*

A437G 5'-CACGGTTGTGGTGTCTGACAAGTTGGG-3' (SEQ ID NO: 75)  
 5'-CCCAACTTGTCGACACCACAACCGTG-3'

5

*Mun I*

A463E 5'-GATCTGGTGGCAATTGGGAGGAATGTTTCG-3' (SEQ ID NO: 76)  
 5'-CGAAACATTCCTCCCAATTGCCACCAGATC-3'

10 and, accordingly, for other mutations.

The temperature optimum of the purified phytases, expressed in *Saccharomyces cerevisiae* (Example 7), was determined as outlined in Example 9. Table 5 shows the effect of  
 15 each mutation introduced on the stability of consensus phytase-1.

Table 5

Stability effect of the individual amino acid replacements in  
 20 consensus phytase-1

+ or - means a positive, respectively, negative effect on the protein stability up to 1°C, ++ and -- means a positive, respectively, negative effect on the protein stability between 1 and 3°C; the numbers 10 or 11 in parentheses indicate the  
 25 consensus phytase sequence that suggested the amino acid replacement.

stabilizing		neutral		destabilizing	
mutation	effect	mutation	effect	mutation	effect
E58A (10)	+	D69A	±	Y54F (10)	-
D69K (11)	+	D70G (10)	±	V73I	-
D197N (10)	+	N134Q (10)	±	A94K (10)	-
T214L (10)	+ +	G186H	±	A101R (11)	-
E222T (11)	+ +	S187A (10)	±	K153N (11)	-
E267D (10)	+	T214V	±	I158V (10)	- -
R291I	+	T251N (10)	±	G203A	- -
R329H (10)	+	Y259N (10)	±	G205S	-
S364T (10)	+ +	A283D (10)	±	A217V	-
A379K (11)	+	A320V (10)	±	V227A (11)	- -
G404A (10)	+ +	K445T	±	L234V (10)	-
		A463E (10)	±	A238P (10)	- -
				E277Q (10)	-
				H287A (11)	-
				Q292A	-
				I366V (10)	-
				S396A (10)	- -
				Q415E (11)	-
				A437G (10)	- -
				E451R	- -

We combined eight positive mutations (E58A, D197N, E267D, R291I, R329H, S364T, A379K, G404A) in consensus phytase-1 thermo[8], using the primers and the technique mentioned above in this example. Furthermore, the mutations Q50T and/or K91A were introduced which mainly influence the catalytic characteristics of phytase (see patent applications EP 897010 and EP 897985, as well as Example 9). The DNA and amino acid sequence of the resulting phytase (consensus phytase-1 thermo[8]-Q50T-K91A) are shown in Figure 7. In this way, the temperature optimum and the melting point of the consensus phytase were increased by 7°C (Figures 15, 16, 17).

In a further consensus protein, we combined eleven positive mutations (E58A, D69K, D197N, T214L, E222T, E267D, R291I, R329H, S364T, A379K, G404A) in consensus phytase-1 thermo[11]. Furthermore, the mutations Q50T and/or K91A were

introduced. In this way, the melting temperature was increased by another 3-4°C when compared to consensus phytase-1 thermo[8].

Using the results of Table 5, we further improved the thermostability of consensus phytase-10 by the back mutations  
5 K94A, V158I, and A396S, the reverse of which (A94K, I158V, and S396A) revealed a strong negative influence on the stability of consensus phytase-1. The resulting protein was called consensus phytase-10-thermo[3]. SEQ ID NO: 26 plus the three mutations K94A, V158I, and A396S. Furthermore, we introduced the mutations  
10 Q50T and K91A that mainly influence the catalytic characteristics of consensus phytase (see patent applications EP 897010 and EP 897985, as well as Example 9 and Figures 14 and 15). The resulting DNA and amino acid sequence are shown in Figure 8. The optimized phytase showed a 4°C higher temperature  
15 optimum and melting point than consensus phytase-10 (Figures 12 and 13). Furthermore, the phytase has also a strongly increased specific activity with phytate as substrate of 250 U/mg at pH 5.5 (Figure 14).

In a still further consensus protein, two additional  
20 mutations were introduced into consensus phytase-10 thermo[3] (E222T, G437A) which yielded consensus phytase-10 thermo[5]. Furthermore, the mutations Q50T and/or K91A were introduced. In this way, the melting temperature was increased by another 1-2°C when compared to consensus phytase-10 thermo[3].

Example 4

Stabilization of the phytase of A. fumigatus ATCC 13073 by replacement of amino acid residues with the corresponding consensus phytase-1 and/or consensus phytase-10 residues

5

At six amino acid sequence positions where A. fumigatus 13073 phytase is the only or nearly the only phytase in the alignment of Figure 1 that does not contain the corresponding consensus phytase amino acid residue, the non-consensus amino acid residue was replaced by the consensus one. The following amino acids were substituted in A. fumigatus 13073 phytase, containing additionally the Q51(24)T substitution (influencing the catalytic properties and corresponding to the Q50T substitution in the consensus phytases) and the signal sequence of A. terreus cbs116.46 phytase (see European Patent Application No. 0897010, and Figure 9): F55(28)Y, V100(73)I, F114(87)Y, A243(220)L, S265(242)P, N294(282)D. The numbers in parentheses refer to the numbering in Figure 1.

In a second round, four of the seven stabilizing amino acid exchanges (E58A, R329H, S364T, G404A) identified in consensus phytase-10 and tested as single mutations in consensus phytase-1 (Table 5) were additionally introduced into the A. fumigatus alpha-mutant. Furthermore, the amino acid replacement S154N, shown to reduce the protease susceptibility of the phytase, was introduced.

The mutations were introduced as described in Example 3 (see Table 6) and expressed as described in Examples 6 to 8. The resulting A. fumigatus 13073 phytase variants were called alpha-mutant (i.e. the A. fumigatus ATCC 13073 phytase with the substitutions Q24T, F28Y, V73I, F87Y, A220L, S242P, N282D) and "optimized" alpha-mutant (i.e. the A. fumigatus alpha-mutant

having the additional substitutions E59A-S154N-R329H-S364T-G404A). K92A is an additional preferred mutation.

The temperature optimum (60°C, Figure 20) and the melting temperature (67.0°C, Figure 19) of the *A. fumigatus* 13073 alpha-  
5 mutant phytase were increased by 5-7°C in comparison to the values of the wild-type phytase (temperature optimum: 55°C, T<sub>m</sub>: 60°C). The five additional amino acid replacements further increased the temperature optimum by 3°C (Figure 20).

# 10 Table 6

Mutagenesis primers for the stabilization of *A. fumigatus* ATCC 13073 phytase

Mutation	Primer
15 F55Y	5' -CACGTACTCGCCATACTTTTCGCTCGAG-3' (SEQ ID NO: 77) 5' -CTCGAGCGAAAAGTATGGCGAGTACGTG-3'
	(Xho I)
E58A	5' -CCATACTTTTCGCTCGCGGACGAGCTGTCCGTG-3' (SEQ ID NO: 78)
20	5' -CACGGACAGCTCGTCCGCGAGCGAAAAGTAGG-3'
V100I	5' -GTATAAGAAGCTTATTACGGCGATCCAGGCC-3' (SEQ ID NO: 79)
25	5' -GGCCTGGATCGCCGTAATAAGCTTCTTATAC-3'
F114Y	5' -CTTCAAGGGCAAGTACGCCTTTTTGAAGACG-3' (SEQ ID NO: 80)
30	5' -CGTCTTCAAAAAGGCGTACTTGCCCTTGAAG-3'
A243L	5' -CATCCGAGCTCGCCTCGAGAAGCATCTTC-3' (SEQ ID NO: 81) 5' -GAAGATGCTTCTCGAGGCGAGCTCGGATG-3'
S265P	5' -CTAATGGA TGTGTCCGTTTGATACGGTAG-3' (SEQ ID NO: 82)
35	5' -CTACCGTATCAAACGGACACATGTCCATTAG-3'
N294D	5' -GTGGAAGAAGTACGACTACCTTCAGTC-3' (SEQ ID NO: 83) 5' -GACTGAAGGTAGTCGTACTTCTTCCAC-3'

(Mlu I)

R329H 5'-GCCCCGGTTGACGCAATTCGCCAGTGCAGG-3' (SEQ ID NO: 84)  
 5'-CCTGCACTGGCGAATGCGTCAACCGGGC-3'

5

Nco I

S364T 5'-CACACGACAACACCATGGTTTCCATCTTC-3' (SEQ ID NO: 85)  
 5'-GAAGATGGAAACCATGGTGTTCGTGTG-3'

(Bss HI)

10 G404A 5'-GTGGTGCCTTTCGCGCGCGAGCCTACTTC-3' (SEQ ID NO: 86)  
 5'-GAAGTAGGCTCGCGCGCGAAAGGCACCAC-3'

Example 5

Introduction of the active site amino acid residues of A.  
 15 niger NRRL 3135 phytase into consensus phytase-1

We used the crystal structure of *Aspergillus niger* NRRL 3135 phytase to define all active site amino acid residues (see Example 1, and EP 897010). Using the alignment of Figure 1, we replaced the following active site residues and additionally  
 20 the non-identical adjacent ones of consensus phytase-1 by those of A. niger phytase:

S89D, S92G, A94K, D164S, P201S, G203A, G205S, H212P, G224A, D226T, E255T, D256E, V258T, P265S, Q292H, G300K, Y305H, A314T, S364G, M365I, A397S, S398A, G404A, and A405S.

25 The new consensus phytase-7 protein sequence was backtranslated into a DNA sequence (Figure 10) as described in Example 1. The corresponding gene (fcp7) was generated as described in Example 1 using the following oligonucleotide mixes:

30 Mix 1.7: CP-1, CP-2, CP-3, CP-4.7, CP-5.7, CP-6, CP-7, CP-8.7, CP-9, CP-10.7

Mix 2.7: CP-9, CP-10.7, CP-11.7, CP-12.7, CP-13.7, CP-14.7, CP-15.7, CP-16, CP-17.7, CP-18.7, CP-19.7, CP-20, CP-21, CP-22.

35

The DNA sequences of the oligonucleotides are indicated in Figure 10. The newly synthesized oligonucleotides are additionally marked by the number 7. After assembling of the 5 oligonucleotides using the same PCR primers as mentioned in Example 1, the gene was cloned into an expression vector as described in Examples 6-8.

The pH-profile of the enzyme determined after expression in *H. polymorpha* and purification was very similar to that of *A. niger* phytase (see Figure 18).

#### Example 6

#### Expression of the consensus phytase genes in *Hansenula polymorpha*

15 The phytase expression vectors used to transform *H. polymorpha* RB11 [Gellissen, G., Hollenberg, C. P., Janowicz, Z. A. (1994) Gene expression in methylotrophic yeasts, in Smith, A. (ed.) Gene expression in recombinant microorganisms. Dekker, New York, pp. 395-439] were constructed by inserting the Eco RI fragment 20 of pBsk-fcp or variants thereof into the multiple cloning site of the *H. polymorpha* expression vector pFPMT121, which is based on an *ura3* selection marker from *S. cerevisiae*, a formate dehydrogenase (FMD) promoter element and a methanol oxidase (MO) terminator element from *H. polymorpha*. The 5' end of the fcp 25 gene is fused to the FMD promoter, the 3' end to the MOX terminator (Gellissen et al., Appl. Microbiol. Biotechnol. 46, 46-54, 1996; EP 299108). The resulting expression vectors are designated pFPMTfcp, pFPMTfcp10, and pFPMTfcp7.

The constructed plasmids were propagated in *E. coli*. 30 Plasmid DNA was purified using standard state of the art procedures. The expression plasmids were transformed into the *H.*

polymorpha strain RB11 deficient in orotidine-5'-phosphate decarboxylase (ura3) using the procedure for preparation of competent cells and for transformation of yeast as described in Gellissen et al. (1996). Each transformation mixture was plated  
5 on YNB medium (0.14% w/v Difco YNB and 0.5% ammonium sulfate) containing 2% glucose and 1.8% agar, and incubated at 37 °C. After 4 to 5 days individual transformant colonies were picked and grown in the liquid medium described above for 2 days at 37 °C. Subsequently, an aliquot of this culture was used to  
10 inoculate fresh vials with YNB-medium containing 2% glucose. After seven further passages in selective medium, the expression vector had integrated into the yeast genome in multimeric form. Subsequently, mitotically stable transformants were obtained by two additional cultivation steps in 3 ml non-selective liquid  
15 medium (YPD, 2% glucose, 10 g/l yeast extract, and 20 g/l peptone). In order to obtain genetically homogeneous recombinant strains, an aliquot from the last stabilization culture was plated on a selective plate. Single colonies were isolated for analysis of phytase expression in YNB containing 2% glycerol  
20 instead of glucose to derepress the FMD promoter. Purification of the consensus phytases was done as described in Example 7.

#### Example 7

Expression of the consensus phytase genes in Saccharomyces  
25 cerevisiae and purification of the phytases from the  
culture supernatant

The consensus phytase genes were isolated from the corresponding Bluescript-plasmid (pBsk-fcp, pBSK-fcp10, pBsk-  
30 fcp7) and ligated into the Eco RI sites of the expression cassette of the Saccharomyces cerevisiae expression vector pYES2

(Invitrogen, San Diego, CA, USA) or subcloned between the shortened GAPFL (glyceraldehyde-3-phosphate dehydrogenase) promoter and the *pho5* terminator as described by Janes et al., Curr. Genet. 18, 97-103. The correct orientation of the gene was  
5 checked by PCR. Transformation of *S. cerevisiae* strains, e.g. INVSc1 (Invitrogen, San Diego, CA, USA), was done according to Hinnen et al., Proc. Natl. Acad. Sci. USA 75, 1929-1933 (1978). Single colonies harboring the phytase gene under the control of the GAPFL promoter were picked and cultivated in 5 ml selection  
10 medium [SD-uracil; Sherman, J. P., Finck, G. R. & Hicks, J. B. (1986) Laboratory course manual for methods in yeast genetics. Cold Spring Harbor University] at 30°C under vigorous shaking (250 rpm) for one day. The preculture was then added to 500 ml YPD medium (Sherman et al., 1986) and grown under the same  
15 conditions. Induction of the *gal1* promoter was done according to the manufacturer's instructions. After four days of incubation, the cell broth was centrifuged (7000 rpm, GS3 rotor, 15 min, 5°C) to remove the cells, and the supernatant was concentrated by way of ultrafiltration in Amicon 8400 cells (PM30 membranes;  
20 Grace AG, Wallizeller, Switzerland) and ultrafree-15 centrifugal filter devices (Biomax-30K, Millipore, Bedford, MA, USA). The concentrate (10 ml) was desalted on a 40 ml Sephadex G25 Superfine column (Pharmacia Biotech, Freiburg, Germany), with 10 mM sodium acetate, pH 5.0, serving as elution buffer. The  
25 desalted sample was brought to 2 M  $(\text{NH}_4)_2\text{SO}_4$  and directly loaded onto a 1 ml Butyl Sepharose 4 Fast Flow hydrophobic interaction chromatography column (Pharmacia Biotech, Freiburg, Germany) which was eluted with a linear gradient from 2 M to 0 M  $(\text{NH}_4)_2\text{SO}_4$  in 10 mM sodium acetate, pH 5.0. Phytase was eluted in the  
30 breakthrough, concentrated and loaded on a 120 ml Sephacryl S-300 gel permeation chromatography column (Pharmacia Biotech,

Freiburg, Germany). Consensus phytases -1, -7 and -10 eluted as a homogeneous symmetrical peak and were shown by SDS-PAGE to be approx. 95% pure.

5        Example 8

Expression of the consensus phytase genes in Aspergillus niger

         The Bluescript-plasmids pBsk-fcp, pBsk-fcp10, and pBsk-fcp7 were used as template for the introduction of a Bsp HI-site upstream of the start codon of the genes and an Eco RV-site downstream of the stop codon. The Expand<sup>TM</sup> High Fidelity PCR Kit (Boehringer Mannheim, Mannheim, Germany) was used with the following primers:

15 Primer Asp-1:

*Bsp HI*

         5'-TATATCATGAGCGTGTTTCGTCGTGCTACTGTTC-3' (SEQ ID NO: 87)

Primer Asp-2 used for cloning of fcp and fcp7:

20

*Eco RV*

         3'-ACCCGACTTACAAAGCGAATTCTATAGATATAT-5' (SEQ ID NO: 88)

Primer Asp-3 used for cloning of fcp10:

25

*Eco RV*

         3'-ACCCTTCTTACAAAGCGAATTCTATAGATATAT-5' (SEQ ID NO: 89)

         The reaction was performed as described by the supplier.  
30 The PCR-amplified fcp-genes had a new Bsp HI site at the start codon, introduced by primer Asp-1, which resulted in a

replacement of the second amino acid residue glycine by serine. Subsequently, the DNA-fragment was digested with Bsp HI and Eco RV and ligated into the Nco I site downstream of the glucoamylase promoter of *Aspergillus niger* (glaA) and the Eco RV site upstream of the *Aspergillus nidulans* tryptophan C terminator (trpC) (Mullaney et al., 1985). After this cloning step, the genes were sequenced to detect possible errors introduced by PCR. The resulting expression plasmids, which basically correspond to the pGLAC vector as described in Example 9 of EP 684313, contained the orotidine-5'-phosphate decarboxylase gene (pyr4) of *Neurospora crassa* as a selection marker. Transformation of *Aspergillus niger* and expression of the consensus phytase genes was done as described in EP 684313. The consensus phytases were purified as described in Example 7.

#### Example 9

##### Determination of phytase activity and of the pH and temperature optima

This example relates i.a. to the determination of phytase activity and of the temperature optimum. Various phytases have been tested.

The phytase of *Aspergillus niger* NRRL 3135 was prepared as described in EP 420358 and by van Hartingsveldt et al. (Gene 127, 87-94, 1993).

The phytases of *Aspergillus fumigatus* ATCC 13073, *Aspergillus terreus* 9A-1, *Aspergillus terreus* cbs116.46, *Emmericella nidulans*, *Myceliophthora thermophila*, and *Talaromyces thermophilus* were prepared as described in EP-0897985 and in the references therein.

The remaining phytases tested were prepared as described herein.

Consensus phytase-1-thermo(8) designates a variant of consensus phytase-1, which further comprises the eight mutations which are underlined in the legend to Figure 5. Consensus phytase-1 is shown in Fig. 1 (SEQ ID NO: 14) without signal peptide, and in Fig. 2 (SEQ ID NO: 16) with the signal peptide.

Phytase activity was determined basically as described by Mitchell et al. (1997). The activity was measured in an assay mixture containing 0.5% phytic acid ( $\approx 5$  mM) in 200 mM sodium acetate, pH 5.0. After 15 min of incubation at 37°C, the reaction was stopped by addition of an equal volume of 15% trichloroacetic acid. The liberated inorganic phosphate was quantified by mixing 100  $\mu$ l of the assay mixture with 900  $\mu$ l H<sub>2</sub>O and 1 ml of 0.6 M H<sub>2</sub>SO<sub>4</sub>, 2% ascorbic acid and 0.5% ammonium molybdate. Standard solutions of potassium phosphate were used as reference. One unit of enzyme activity was defined as the amount of enzyme that releases 1  $\mu$ mol phosphate per minute at 37°C. The protein concentration was determined using the enzyme extinction coefficient at 280 nm calculated according to Pace et al. [Pace N. C., Vajdos, F., Fee, L., Grimsley, G. & Gray, T. (1995) How to measure and predict the molar absorption coefficient of a protein. Prot. Sci. 4, 2411-2423]: 1 absorption unit (1 OD) at 280 nm corresponds to 1.101 mg/ml of consensus phytase-1, 1.068 mg/ml of consensus phytase-7, and 1.039 mg/ml of consensus phytase-10.

In case of pH-optimum curves, the purified enzymes were diluted in 10 mM sodium acetate, pH 5.0. Incubations were started by mixing aliquots of the diluted protein with an equal volume of 1% phytic acid ( $\approx 10$  mM) in a series of different buffers: 0.4 M glycine/HCl, pH 2.5; 0.4 M acetate/NaOH, pH 3.0, 3.5, 4.0, 4.5, 5.0, 5.5; 0.4 M imidazole/HCl, pH 6.0, 6.5; 0.4 M Tris/HCl pH 7.0, 7.5, 8.0, 8.5, 9.0. Control experiments showed

that pH was only slightly affected by the mixing step. Incubations were performed for 15 min at 37°C as described above.

For determination of the substrate specificities of the 5 phytases, phytic acid in the assay mixture was replaced by 5 mM concentrations of the respective phosphate compounds. Besides, the activity tests were performed as described above.

For determination of the temperature optimum, enzyme (100  $\mu$ l) and substrate solution (100  $\mu$ l) were pre-incubated for 5 min 10 at the given temperature. The reaction was started by addition of the substrate solution to the enzyme. After 15 min of incubation, the reaction was stopped with trichloroacetic acid, and the amount of phosphate released was determined.

The pH-optimum of consensus phytase-1 was around pH 6.0- 15 6.5 (70 U/mg). Introduction of the Q50T mutation shifted the pH-optimum to pH 6.0 (130 U/mg). Introduction of the K91A mutation further shifted the pH optimum into the more acidic pH-range. Comparable effects of the Q50T and K91A mutations were also observed for consensus phytase-10 and for further 20 stabilized consensus phytase variants (Figures 14 and 15).

Consensus phytase-7, which was constructed to transfer the catalytic characteristics of *A. niger* NRRL 3135 phytase to consensus phytase-1, had a pH-profile very similar to that of *A. niger* NRRL 3135 phytase (see Figure 18). The substrate 25 specificity also resembled more that of *A. niger* NRRL 3135 phytase than that of consensus phytase-1.

The temperature optimum of consensus phytase-1 (71°C) was 16-26°C higher than the temperature optima of the wild-type phytases (45-55°C, Table 7) that were used to calculate the 30 consensus sequence. The improved consensus phytase-10 showed a further increase of its temperature optimum to 80°C (Figure 13).

The temperature optimum of consensus phytase-1-thermo[8] was found to be in the same range (78°C) when using the supernatant of an overproducing *S. cerevisiae* strain. The highest temperature optimum reached of 82°C was determined for consensus phytase-10-thermo[3]-Q50T-K91A. Table 7

Temperature optima and T<sub>m</sub>-values of consensus phytase and of the phytases from *A. fumigatus*, *A. niger*, *E. nidulans*, and *M. thermophila*.

10

The determination of the temperature optimum was performed as described in Example 9. The T<sub>m</sub>-values were determined by differential scanning calorimetry as described in Example 10.

Phytase	Optimum temperature (°C)	T <sub>m</sub> (°C)
<i>Aspergillus niger</i> NRRL 3135	55	63.3
<i>Aspergillus fumigatus</i> ATCC 13073	55	62.5
<i>Aspergillus terreus</i> 9A-1	49	57.5
<i>Aspergillus terreus</i> cbs116.46	45	58.5
<i>Emmericella nidulans</i>	45	55.7
<i>Myceliophthora thermophila</i>	55	-
<i>Talaromyces thermophilus</i>	45	-
Consensus phytase- 10-thermo[5]-Q50T- K91A	-	90.4
Consensus-phytase- 10-thermo[3]-Q50T- K91A	82	89.3

	55	
Consensus-phytase-10-thermo[3]-Q50T	82	88.6
Consensus-phytase-10	80	85.4
Consensus phytase-1-thermo[11]-Q50T-K91A	-	88.0
Consensus phytase-1-thermo[11]-Q50T	-	88.5
Consensus-phytase-1-thermo[8]-Q50T-K91A	-	85.7
Consensus-phytase-1-thermo[8]-Q50T	78	84.7
Consensus-phytase-1-thermo[8]	81	-
Consensus-phytase-1-thermo[3]	75	-
Consensus-phytase-1-Q50T	-	78.9
Consensus-phytase-1	71	78.1
Aspergillus fumigatus $\alpha$ -mutant Q51T	60	67.0
Aspergillus fumigatus $\alpha$ -mutant, plus mutations E59A, S154N, R329H, S364T, G404A	63	-
Aspergillus fumigatus "optimized" $\alpha$ -mutant, plus mutation K92A	63	-

Example 10Determination of the melting temperature by differential scanning calorimetry (DSC)

5 In order to determine the unfolding temperature of the phytases, differential scanning calorimetry was applied as described by Brugger et al., 1997 [Brugger, R., Mascarello, F., Augem, S., van Loon, A. P. G. M. & Wyss, M. (1997). Thermal denaturation of phytases and pH 2.5 acid phosphatase studied by  
10 differential scanning calorimetry. In The Biochemistry of phytate and phytase (eds. Rasmussen, S.K.; Raboy, V.; Dalbøge, H. and Loewus, F.; Kluwer Academic Publishers, Dordrecht, the Netherlands]. Solutions of 50-60 mg/ml of homogeneous phytase were used for the tests. A constant heating rate of 10°C/min was applied up to 90-95°C.  
15

The determined melting points confirm the results obtained for the temperature optima (Table 7). The most stable consensus phytase designed so far is consensus phytase-10-thermo[3]-Q50T-K91A showing a melting temperature under the chosen conditions  
20 of 89.3°C. This is 26.0 to 33.6°C higher than the melting temperature of the wild-type phytases used.

Example 11Transfer of basidiomycete phytase active site into  
25 consensus phytase-10-thermo[3]-Q50T-K91A

As described previously (Example 5), mutations derived from the basidiomycete phytase active sites were introduced into consensus phytase-10. The following five constructs a) to e) were prepared:

30 a) The construct called consensus phytase-12, and it comprises a selected number of active site residues of the

basidio consensus sequence. Its amino acid sequence is shown in Fig. 21 (the first 26 amino acids form the signal peptide; positions differing from consensus phytase-10-thermo[3]-Q50T-K91A are underlined);

5        b) a cluster of mutations (Cluster II) was transferred to the consensus phytase-1 and -10 sequences, viz.: S80Q, Y86F, S90G, K91A, S92A, K93T, A94R, Y95I;

      c) in a similar way, another cluster of mutations (Cluster III) was transferred, viz.: T129V, E133A, Q134N, M136S, V137S,  
10 N138Q, S139A;

      d) in a similar way, a further cluster of mutations (Cluster IV) was transferred, viz.: A168D, E171T, K172N, F173W;

      e) and finally, a further cluster of mutations (Cluster V) was transferred, viz.: Q297G, S298D, G300D, Y305T.

15        These constructs were expressed as described in Examples 6 to 8.

#### Example 12

##### Phytase alignment using GAP

20        The phytases described herein - i.e. the amino acid sequences as well as the corresponding DNA sequences - were aligned against each other. Also some other phytases were correspondingly aligned, viz. the following:

- the consensus phytase-1 described in EP 897985;
- 25 - the phytase derived from *Aspergillus niger* (ficuum) NRRL 3135 (A. niger NRRL3135) described in EP 420358;
- the phytases derived from *Aspergillus fumigatus* ATCC 13073 (A. fumigatus 13073); *Aspergillus fumigatus* ATCC 32239 (A. fumigatus 32239); *Aspergillus terreus* cbs116.46 (A. terreus cbs);
- 30 *Emericella nidulans* (E. nidulans); and *Talaromyces thermophilus* (T. thermophilus) - all described in EP 897010;

- the phytases derived from *Myceliophthora thermophila* (*M. thermophila*); and *Aspergillus terreus* 9-A1 (*A. terreus* 9-A1) - both described in EP 684313;
- the phytase derived from *Thermomyces lanuginosus* (*T. lanuginosus*) described in WO 9735017 (PCT/US97/04559);
- the phytases derived from *Agrocybe pediades* (*A. pediades*), *Paxillus involutus* 1 and 2 (*P. involutus* phyA1 and phyA2); and *Trametes pubescens* (*T. pubescens*) - all described in WO 98/28409; and
- 10 - the phytase derived from *Peniophora lycii* (*P. lycii*) described in WO 98/28408.

For the alignments, the program GAP was used with the settings as described above.

For polypeptide comparisons, the signal peptides were 15 included with the exception of comparisons with consensus phytase-11.

The results of the amino acid sequence comparisons are shown in Table 8 below. The first number in each cell is the amino acid similarity, the second number is the amino acid 20 identity.

For DNA sequence comparisons, the signal sequence was always included. The results are shown in Table 9 below.

This invention comprises e.g. the following embodiments (A) to (J) that are described below.

25 In these embodiments, when determining % identity or % similarity at the amino acid level for another phytase, its amino acid sequence is aligned with the reference sequence (e.g. in embodiment (A) the consensus phytase-10 amino acid sequence), using an alignment program such as GAP referred to above.  
30 Percentage identity, as well as percentage similarity, is

calculated by the program. The amino acid sequence of the other phytase may or may not include the signal peptide.

When determining % identity on the DNA level for another phytase-encoding DNA, this DNA sequence is aligned with the  
5 reference sequence [e.g. in embodiment (A) nucleotides 12-1412 of SEQ ID NO: 25 (the DNA sequence of consensus phytase-10 (Fcp10) as shown in Fig. 5)], using an alignment program such as GAP referred to above. Percentage identity is calculated by the program. The DNA sequence encoding the other phytase can be a  
10 genomic DNA sequence including introns, or it can be a cDNA sequence. It may or may not include the signal peptide-encoding part.

When determining hybridization, the probe to be used is the specified DNA sequence [e.g. in embodiment (A) nucleotides  
15 12-1412 of SEQ ID NO: 25 (the DNA sequence of consensus phytase-10 (Fcp10) as shown in Fig. 5)]. The DNA sequence encoding the other phytase can be a genomic DNA sample which contains a phytase-encoding DNA-sequence; a purified genomic DNA sequence (purified with respect to the phytase-encoding DNA sequence); or  
20 it can be a phytase-encoding cDNA sequence, preferably purified or amplified, e.g. PCR-amplified. The phytase-encoding DNA, whatever type, may or may not include the signal peptide-encoding part. Suitable hybridization conditions are referred to above.

25 The term "DNA sequence" includes such fragments or parts of the herein exemplified DNA sequences, as long as they are capable of encoding an active enzyme (e.g. phytase).

The term "amino acid sequence" includes such fragments or parts of the herein exemplified amino acid sequences, as long  
30 as they are enzymatically active (e.g. displaying phytase activity).

(A) Phytases and corresponding DNA sequences related to consensus phytase-10 (CP10, Fcp 10)

A phytase that comprises an amino acid sequence which is at least 93.80%; or at least 94, 94.5, 95, 95.5, 96, 96.5, 97, 5 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-10 (Fcp10) as shown in Fig. 5.

A phytase that comprises an amino acid sequence which is at least 95.09%; or at least 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of 10 consensus phytase-10.

A phytase which is encoded by a DNA sequence which is at least 95.88%; or at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase-10 (Fcp10) as shown in Fig. 5.

15 A DNA sequence which encodes a phytase and which (i) is at least 95.88%; or at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical; or (ii) hybridizes under low, or medium, medium/high, high, or very high stringency conditions to nucleotides 12-1412 of the DNA sequence of consensus phytase-10 20 (Fcp10) as shown in Fig. 5. A suitable negative control is DNA encoding consensus phytase-1. A suitable positive control is DNA encoding any of CP10, CP10-thermo[3]-Q50T, K91A, CP1-thermo[8], CP1-thermo[8]Q50T, K91A.

A DNA sequence which encodes a phytase comprising an amino 25 acid sequence which is at least 93.80%; or at least 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-10 (Fcp10) as shown in Fig. 5.

(B) Phytases and corresponding DNA sequences related to  
consensus phytase-10-thermo[3]-Q50T-K91A

A phytase which comprises an amino acid sequence which is at least 93.37%; or at least 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 5 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-10-thermo[3]-Q50T-K91A as shown in Fig. 8.

A phytase which comprises an amino acid sequence which is at least 94.66%; or at least 95.0, 95.5, 96, 96.5, 97, 97.5, 98, 10 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase-10-thermo[3]-Q50T-K91A as shown in Fig. 8.

A phytase which is encoded by a DNA sequence which is at least 95.88%; or at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of 15 consensus phytase-10-thermo[3]-Q50T-K91A as shown in Fig. 8.

A DNA sequence which encodes a phytase and which (i) is at least 95.88%; or at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical; or (ii) hybridizes under low, or medium, medium/high, high, or very high stringency conditions to 20 nucleotides 12-1412 of the DNA sequence of consensus phytase-10-thermo[3]-Q50T-K91A as shown in Fig. 8. A suitable negative control is DNA encoding consensus phytase-1. A suitable positive control is DNA encoding any of CP10, CP10-thermo[3]-Q50T-K91A, CP1-thermo[8], or CP1-thermo[8]-Q50T-K91A.

25 A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 93.37%; or at least 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-10-thermo[3]-Q50T-K91A as shown in Fig. 8.

(C) Phytases and corresponding DNA sequences related to  
consensus phytase-1-thermo[8]

A phytase which comprises an amino acid sequence which is at least 98.30%; or at least 98.5, 99, 99.5% identical to the  
5 sequence of amino acids 1-467 of consensus phytase-1-thermo[8] (as shown in Fig. 7; backmutations T50Q and A91K to be added).

A phytase which comprises an amino acid sequence which is at least 98.51%; or at least 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase-1-thermo[8] (as shown  
10 in Fig. 7; backmutations T50Q and A91K to be added).

A phytase which is encoded by a DNA sequence which is at least 98.73%; or at least 99, 99.5% identical to nucleotides 1-1407 of the DNA sequence of consensus phytase-1-thermo[8] (as shown in Fig. 7; backmutations T50Q and A91K to be added).

15 A DNA sequence which encodes a phytase and which (i) is at least 98.73%; or at least 99, 99.5% identical; or (ii) hybridizes under low, or medium, medium/high, high, or very high stringency conditions to nucleotides 1-1407 of the DNA sequence of consensus phytase-1-thermo[8] (as shown in Fig. 7;  
20 backmutations T50Q and A91K to be added). A suitable negative control is DNA encoding consensus phytase-1. A suitable positive control is DNA encoding any of CP1-thermo[8], CP1-thermo[8]-Q50T-K91A.

A DNA sequence which encodes a phytase comprising an amino  
25 acid sequence which is at least 98.30%; or at least 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-1-thermo[8] (as shown in Fig. 7; backmutations T50Q and A91K to be added).

(D) Phytases and corresponding DNA sequences related to consensus phytase-1-thermo[8]-Q50T-K91A

A phytase which comprises an amino acid sequence which is at least 97.87%; or at least 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-1-thermo[8]-Q50T-K91A as shown in Fig. 7.

A phytase which comprises an amino acid sequence which is at least 98.08%; or at least 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase-1-thermo[8]-Q50T-K91A as shown in Fig. 7.

A phytase which is encoded by a DNA sequence which is at least 98.37%; or at least 98.5, 99, 99.5% identical to nucleotides 1-1407 of the DNA sequence of consensus phytase-1-thermo[8]-Q50T-K91A as shown in Fig. 7.

A DNA sequence which encodes a phytase and which (i) is at least 98.37%; or at least 98.5, 99, 99.5% identical; or (ii) hybridizes under low, or medium, medium/high, high, or very high stringency conditions to nucleotides 1-1407 of the DNA sequence of consensus phytase-1-thermo[8]-Q50T-K91A as shown in Fig. 7. A suitable negative control is DNA encoding consensus phytase-1. A suitable positive control is DNA encoding any of CP1-thermo[8], CP1-thermo[8]-Q50T-K91A.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 97.87%; or at least 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase 1-thermo[8]-Q50T-K91A as shown in Fig. 7.

(E) Phytases and corresponding DNA sequences related to consensus phytase-11

A phytase that comprises an amino acid sequence which is at least 90.71%; or at least 91, 91.5, 92, 92.5, 93, 93.5, 94,

94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-482 of consensus phytase-11 as shown in Fig. 6.

A phytase that comprises an amino acid sequence which is at least 92.07%; or at least 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-482 of consensus phytase-11 as shown in Fig. 6.

A DNA sequence that encodes a phytase comprising an amino acid sequence which is at least 90.71%; or at least 91, 91.5, 92, 92.5, 93, 93.5, 94, 94.5, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-482 of consensus phytase-11 as shown in Fig. 6.

(F) Phytases and corresponding DNA sequences related to A. fumigatus alpha-mutant

A phytase that comprises an amino acid sequence which is at least 97.17%; or at least 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of A. fumigatus alpha-mutant (phytase) as shown in Fig. 9.

A phytase that comprises an amino acid sequence that is at least 97.82%; or at least 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of A. fumigatus alpha-mutant (phytase) as shown in Fig. 9.

A phytase which is encoded by a DNA sequence which is at least 96.13%; or at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 1-1401 of the DNA sequence of A. fumigatus ATCC 13073 alpha-mutant (phytase) as shown in Fig. 9.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 97.17%; or at least 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467

of *A. fumigatus* ATCC 13073 alpha-mutant (phytase) as shown in Fig. 9.

A DNA sequence which encodes a phytase and which (i) is at least 96.13%; or 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical; 5 or (ii) hybridizes under low, or medium, medium/high, high, or very high stringency conditions to nucleotides 1-1401 of the DNA sequence of *A. fumigatus* ATCC 13073 alpha-mutant (phytase) shown in Fig. 9. A suitable negative control is DNA encoding *A. fumigatus* 13073 phytase. A suitable positive control is DNA 10 encoding any one of the *A. fumigatus* ATCC 13073 alpha mutant phytase or the optimised alpha-mutant.

(G) Phytases and corresponding DNA sequences related to the optimized *A. fumigatus* alpha-mutant

15 A phytase that comprises an amino acid sequence that is at least 96.08%; or at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of the phytase of the optimized *A. fumigatus* alpha-mutant.

A phytase that comprises an amino acid sequence that is at 20 least 96.74%; or at least 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of the phytase of the optimized *A. fumigatus* alpha-mutant.

A phytase which is encoded by a DNA sequence which is at least 95.63%; or at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 25 99.5% identical to nucleotides 1-1401 of the DNA sequence encoding the optimized *A. fumigatus* alpha-mutant phytase.

A DNA sequence that encodes a phytase comprising an amino acid sequence that is at least 96.08%; or at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the optimized *A. 30 fumigatus* alpha-mutant phytase.

A DNA sequence which encodes a phytase and which (i) is at least 95.63%; or at least 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical; or (ii) hybridizes under low, or medium, medium/high, high, very high stringency conditions to 5 nucleotides 1-1401 of the DNA sequence encoding the optimized *A. fumigatus* alpha-mutant phytase.

A suitable negative control is DNA encoding *A. fumigatus* ATCC 13073 phytase. A suitable positive control is DNA encoding any one of the *A. fumigatus* ATCC 13073 alpha mutant phytase of 10 the optimised alpha-mutant.

(H) Phytases and corresponding DNA sequences related to consensus phytase-7

A phytase that comprises an amino acid sequence which is 15 at least 94.87%; or at least 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-7 as shown in Fig. 10.

A phytase that comprises an amino acid sequence which is at least 95.30%; or at least 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 20 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase-7 as shown in Fig. 10.

A phytase which is encoded by a DNA sequence which is at least 96.38%; or 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase- 25 7 shown in Fig. 10.

A DNA sequence which encodes a phytase and which (i) is at least 96.38%; or at least 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical; or (ii) hybridizes under low, or medium, medium/high, high, or very high stringency conditions to nucleotides 12-1412 30 of the DNA sequence of consensus phytase-7 as shown in Fig. 10.

A DNA sequence which encodes a phytase comprising an amino acid sequence which is at least 94.87%; or at least 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-7 as shown in 5 Fig. 10.

(I) Phytases related to basidio consensus phytase

A phytase which comprises an amino acid sequence which is at least 76.23%; or at least 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 94.5, 95, 95.5, 96, 96.5, 10 97, 97.5, 98, 98.5, 99, 99.5% identical to the combined sequence of (i) amino acids 1-441 of basidio consensus phytase shown in Fig. 3, and (ii) amino acids 1-26 shown in Fig. 5 (the sequence of (ii) to be added at the N-terminus of the sequence of (i)).

A phytase that comprises an amino acid sequence which is 15 at least 79.50%; or at least 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-441 of basidio consensus phytase as shown in Fig. 3.

(J) Phytases related to consensus phytase-12

20 A phytase which comprises an amino acid sequence which is at least 70, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% identical to the sequence of amino acids 1-467 of consensus phytase-12 as shown in Fig. 21.

25 A phytase which comprises an amino acid sequence which is at least 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 95.5, 96, 96.5, 97, 97.5, 98, 98.5, 99, 99.5% similar to the sequence of amino acids 1-467 of consensus phytase-12 as shown in Fig. 21.

Table 8  
Comparison of phytase amino acid sequences

Phytase	CP10	CP10-thermo[3]-Q50T-K91A	CP1-thermo[8]	CP1-thermo[8]-Q50T-K91A	CP11	CP7	Basidio	A. fumigatus alpha-mutant	A. fumigatus alpha-mutant (opt.)
Consensus phytase-1	95.08/93.79	94.65/93.36	98.50/98.29	98.07/97.86	92.06/90.70	95.29/94.86	69.42/62.16	85.59/82.58	84.73/81.72
A. niger NRRL3135	79.48/76.46	79.05/76.03	80.35/77.75	79.91/77.32	79.27/76.31	84.02/81.64	67.19/59.32	74.07/70.11	74.95/70/99
A. terreus 9-A1	76.04/72.11	75.82/71.90	76.47/72.33	76.25/72.11	76.51/73.02	75.76/71.18	65.39/58.02	69.67/64.84	69.45/64.84
A. terreus cbs	79.04/75.11	78.82/74.89	79.48/75.76	79.26/75.55	77.19/73.27	79.17/75.00	66.92/59.65	72.59/67.76	72.37/67.76
E. nidulans	78.70/74.35	78.26/73.91	79.78/75.87	79.35/75.44	80.56/76.62	76.96/73.04	67.20/58.13	72.39/67.83	72.11/67.54
A. fumigatus 13073	82.93/80.31	82.50/79.87	82.31/79.04	81.88/78.60	81.36/78.64	80.13/76.20	63.54/57.91	97.82/97.16	96.73/96.07
A. fumigatus 32239	81.30/77.39	80.87/76.96	81.09/77.61	80.65/77.17	79.95/76.08	79.13/75.22	63.61/54.97	90.22/86.52	89.57/85.87
T. thermophilus	77.83/73.84	77.38/73.39	78.67/74.89	78.22/74.44	78.47/74.76	76.51/73.15	61.54/54.36	72.01/66.82	72.69/67.49
M. thermophila	69.16/62.81	69.48/63.33	69.27/62.84	69.59/63.36	69.65/63.06	68.82/62.13	65.56/57.91	66.21/58.45	66.44/58.68
T. lanuginosus	73.52/66.70	73.06/66.44	71.92/64.61	71.46/64.16	74.21/68.86	69.50/62.62	67.20/57.41	68.91/61.02	69.61/61.72
P. lycii	64.92/59.10	64.91/59.37	64.46/58.09	64.46/58.36	65.03/59.84	63.13/56.50	77.75/73.07	64.08/57.11	62.47/55.91
A. pediades	64.51/51.81	64.86/51.94	62.98/51.41	63.33/51.54	64.50/52.30	63.05/51.15	78.92/74.71	61.64/52.38	62.13/53.07
P. involutus 1	66.67/58.07	66.67/58.33	64.84/56.51	64.84/56.77	63.30/54.52	65.33/56.53	79.49/76.22	59.59/51.81	59.95/52.20
P. involutus 2	65.54/55.70	65.30/55.53	66.85/56.87	66.58/56.68	66.30/56.35	64.27/54.13	78.09/74.59	61.26/52.62	61.04/52.47

T. pubescens	65.46/57.22	65.72/57.47	62.89/55.67	63.14/55.93	65.03/57.65	63.28/56.51	78.34/75.12	64.08/57.11	62.30/55.24
CP10	-	99.57/99.57	96.57/95.50	96.15/95.08	95.02/94.56	91.01/89.29	70.22/62.28	85.13/82.76	85.99/83.62
CP10t[3]Q50TK91A	99.57/99.57	-	96.15/95.08	96.57/95.50	94.56/94.10	90.58/88.87	70.47/62.28	85.13/82.76	85.99/83.62
CP1thermo[8]	96.57/95.50	96.15/95.08	-	99.57/99.57	93.42/92.29	94.43/93.79	68.40/60.74	84.52/81.94	85.38/82.80
CP1t[8]Q50TK91A	96.15/95.08	96.57/95.50	99.57/99.57	-	92.97/91.84	94.00/93.36	68.64/60.74	84.52/81.94	85.38/82.80
CP11	95.02/94.56	94.56/94.10	93.42/92.29	92.97/91.84	-	88.44/86.62	68.27/59.73	82.23/79.73	83.37/80.87
CP7	91.01/89.29	90.58/88.87	94.43/93.79	94.00/93.36	88.44/86.62	-	69.80/62.69	81.94/78.71	81.72/78.50
Basidio	70.22/62.28	70.47/62.28	68.40/60.74	68.64/60.74	68.27/59.73	69.80/62.69	-	65.97/60.52	66.41/60.68
A.fumigatus alpha-mut.	85.13/82.76	85.13/82.76	84.52/81.94	84.52/81.94	82.23/79.73	81.94/78.71	65.97/60.52	-	98.93/98.93
A. fum alpha-mut - opt.	85.99/83.62	85.99/83.62	85.38/82.80	85.38/82.80	83.37/80.87	81.72/78.50	66.41/60.68	98.93/98.93	-

Table 9

## Comparison of phytase encoding DNA sequences

Phytase	CP10	CP10-thermo[3]-Q50T-K91A	CP1-thermo[8]	CP1-thermo[8]-Q50T-K91A	CP7	Basidio	A. fumigatus alpha-mutant	A. fumi-gatus alpha-mutant (opt.)
Consensus phytase-1	95.87	95.87	98.72	98.36	96.37	65.46	66.88	66.88
A. niger NRRL3135	65.10	64.82	66.10	65.74	67.52	50.68	65.88	66.17
A. terreus 9-A1	61.74	61.53	62.17	62.03	60.53	49.40	66.24	66.31
A. terreus cbs	62.52	62.30	63.02	62.88	61.45	49.74	68.17	68.24
E. nidulans	65.08	64.94	65.30	65.01	64.22	49.92	64.90	65.44
A. fumigatus 13073	65.66	65.38	64.19	64.08	63.65	48.27	96.12	95.62
T. thermophilus	62.52	62.50	62.53	62.66	62.00	52.19	61.77	61.92
M. thermophila	55.51	55.15	55.36	55.22	53.91	48.44	58.17	58.24
T. lanuginosus	57.56	57.20	56.76	56.47	62.00	44.66	59.71	60.07
P. lycii	45.76	46.51	45.14	55.21	55.46	58.50	48.91	49.44
A. pediades	49.89	49.89	49.89	50.11	45.54	61.66	47.49	47.56
P. involutus 1	48.32	49.03	47.81	47.96	49.59	59.80	49.96	50.19
P. involutus 2	48.24	49.00	48.08	48.63	47.94	60.16	47.56	47.63
T. pubescens	47.00	47.17	46.46	47.62	46.83	60.37	49.89	49.96
CP10	-	99.43	96.40	96.05	93.73	66.40	67.81	68.24

CP10t [3]Q50TK91A	99.43	-	96.37	96.58	93.45	66.29	67.81	68.24
Cp1thermo [8]	96.40	96.37	-	99.65	95.30	65.40	66.74	67.17
CP1t [8]Q50TK91A	96.05	96.58	99.65	-	94.94	65.47	66.74	67.17
CP7	93.73	93.45	95.30	94.94	-	64.56	65.88	65.88
Basidio	66.40	66.29	65.40	65.47	64.56	-	50.41	50.49
A.fumigatus alpha-mut.	67.81	67.81	66.74	66.74	65.88	50.41	-	99.50
A. fum alpha-mut - opt.	68.24	68.24	67.17	67.17	65.88	50.49	99.50	-

## CLAIMS

1. A phytase that comprises an amino acid sequence which is at least 93.80% identical to the sequence of amino acids 1-467 of consensus phytase-10 (SEQ ID NO: 26).

2. A phytase that is encoded by a DNA sequence that is at least 95.88% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase-10 (SEQ ID NO: 25).

10

3. A phytase that comprises an amino acid sequence chosen from

(i) SEQ ID NO: 26, or amino acids 1-438 thereof; or an amino acid sequence encoded by

15 (ii) nucleotides 12-1412, or 90-1412 of SEQ ID NO: 25.

4. A phytase that comprises an amino acid sequence chosen from

(i) consensus phytase-10-thermo[3],

20 (ii) variants of (i), further including the mutations Q50T, K91A, or (Q50T+K91A), the latter variant being shown in Fig. 8,

(iii) amino acids 27-467 of any of the sequences of (i) and (ii),

25 (iv) SEQ ID NO: 31, or amino acids 1-441 thereof; or an amino acid sequence encoded by

(v) nucleotides 1-1401, or 79-1401 of SEQ ID NO: 30.

5. A phytase that comprises

30 an amino acid sequence chosen from

(i) consensus phytase-1-thermo[8],

- (ii) variants of (i), further including the mutations Q50T, K91A, or (Q50T+K91A), the latter variant being shown in Fig. 7,
- (iii) amino acids 27-467 of any of the sequences of (i) and (ii), or
- (iv) SEQ ID NO: 29, or amino acids 1-441 thereof; or an amino acid sequence encoded by
- (v) nucleotides 1-1407, or 79-1407 of SEQ ID NO: 28.

6. A phytase that comprises the amino acid sequence of consensus phytase-11 (SEQ ID NO: 27).

7. A DNA sequence that comprises a DNA-sequence encoding a phytase of any one of claims 1-6.

15

8. A DNA sequence that comprises a DNA-sequence encoding a phytase, and wherein the phytase-encoding DNA-sequence is (i) at least 95.88% identical, or (ii) hybridizes under high stringency conditions, to nucleotides 12-1412 of the DNA sequence of consensus phytase-10 (SEQ ID NO: 25).

9. A DNA sequence that comprises a DNA-sequence that encodes a phytase, wherein the phytase comprises an amino acid sequence that is at least 93.80% identical to the sequence of amino acids 1-467 of consensus phytase 10 (SEQ ID NO: 26).

10. A DNA sequence that comprises a DNA-sequence that encodes a phytase, and wherein the phytase-encoding DNA-sequence comprises

- (i) nucleotides 12-1412, or 90-1412 of the DNA sequence of consensus phytase-10 (SEQ ID NO: 25);

30

- (ii) nucleotides 1-1401, or 79-1401 of the DNA sequence of consensus phytase-10-thermo[3]-Q50T-K91A (SEQ ID NO: 30); or
- (iii) nucleotides 1-1401, or 79-1401 of the DNA sequence of consensus phytase-1-thermo[8]-Q50T-K91A (SEQ ID NO: 28).

11. A vector comprising a DNA sequence according to any one of claims 7-10.

12. A microbial host cell comprising a DNA sequence according to any one of claims 7-10, or the vector according to claim 11.

13. A process for producing a phytase, the process comprising culturing the host cell according to claim 12 under conditions permitting the production of the phytase, and recovering the phytase from the culture broth.

14. A food, feed or pharmaceutical composition comprising a phytase of any one of claims 1-6.

**AMENDED CLAIMS**

[received by the International Bureau on 16 June 2000 (16.06.00);  
original claims 1 to 14 replaced by new claims 1 to 14 (3 pages)]

1. A phytase that comprises an amino acid sequence which is at least 93.80% identical to the sequence of amino acids -26 to  
5 +441 of consensus phytase 10 (SEQ ID NO: 26).

2. A phytase that is encoded by a DNA sequence that is at least 95.88% identical to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (SEQ ID NO: 25).

10

3. A phytase that comprises  
an amino acid sequence chosen from

(i) SEQ ID NO: 26, or amino acids 1-438 thereof; or  
an amino acid sequence encoded by

15 (ii) nucleotides 12-1412, or 90-1412 of SEQ ID NO:25.

4. A phytase that comprises  
an amino acid sequence chosen from

(i) consensus phytase-10-thermo[3],

20 (ii) variants of (i), further including the mutations  
Q50T, K91A, or (Q50T+K91A), the latter variant  
being shown in Fig. 8,

(iii) amino acids 27-467 of any of the sequences of (i)  
and (ii),

25 (iv) SEQ ID NO: 31, or amino acids 1-441 thereof; or  
an amino acid sequence encoded by

(v) nucleotides 1-1401, or 79-1401 of SEQ ID NO:30.

5. A phytase that comprises

30 an amino acid sequence chosen from

(i) consensus phytase-1-thermo[8],

(ii) variants of (i), further including the mutations  
Q50T, K91A, or (Q50T+K91A), the latter variant  
being shown in Fig. 7,

**AMENDED SHEET (ARTICLE 19)**

(iii) amino acids 27-467 of any of the sequences of (i) and (iv), or

(iv) SEQ ID NO: 29, or amino acids 1-441 thereof; or an amino acid sequence encoded by

5 (v) nucleotides 1-1401, or 79-1401 of SEQ ID NO: 28.

6. A phytase that comprises the amino acid sequence of consensus phytase-11 (SEQ ID NO: 27).

10 7. A DNA sequence that comprises a DNA-sequence encoding the phytase of any one of claims 1-6.

8. A DNA sequence that comprises a DNA-sequence encoding a phytase, and wherein the phytase-encoding DNA-sequence is (i) 15 at least 95.88% identical, or (ii) hybridizes under high stringency conditions, to nucleotides 12-1412 of the DNA sequence of consensus phytase 10 (SEQ ID NO: 25).

9. A DNA sequence that comprises a DNA-sequence that encodes a 20 phytase, wherein the phytase comprises an amino acid sequence that is at least 93.80% identical to the sequence of amino acids -26 to + 441 of consensus phytase 10 (SEQ ID NO: 26).

10. A DNA sequence that comprises a DNA-sequence that encodes 25 a phytase, and wherein the phytase-encoding DNA-sequence comprises

(i) nucleotides 12-1412, or 90-1412 of the DNA sequence of consensus phytase 10 (SEQ ID NO: 25);

30 (ii) nucleotides 1-1401, or 79-1401 of the DNA sequence of consensus phytase-10-thermo[3]-Q50T, K91A (SEQ ID NO: 30); or

(iii) nucleotides 1-1401, or 79-1401 of the DNA sequence of consensus phytase-1-thermo[8]-Q50T, K91A (SEQ ID NO: 28).

11. A vector comprising the DNA sequence according to any one of claims 7-10.

5 12. A microbial host cell comprising the DNA sequence according to any one of claims 7-10, or the vector according to claim 6.

13. A process for producing a phytase, the process comprising  
10 culturing the host cell according to claim 12 under conditions permitting the production of the phytase, and recovering the phytase from the culture broth.

14. A food, feed or pharmaceutical composition comprising the  
15 phytase of any one of claims 1-6.

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	1				50
<i>A. terreus</i> 9A-1	KhsDCNSVDh	GYQCFPELSH	kwGLYAPYFS	LQDESPFPID	VPEDChITFV
<i>A. terreus</i> cbs	NhsDCTSVDr	GYQCFPELSH	kwGLYAPYFS	LQDESPFPID	VPDDChITFV
<i>A. niger</i> var. <i>awamori</i>	NqsTCDTVdQ	GYQCFSETSH	LWGQYAPFFS	LANESAISPD	VPAGCrVTFA
<i>A. niger</i> T213	NqsSCDTVdQ	GYQCFSETSH	LWGQYAPFFS	LANESVISPD	VPAGCrVTFA
<i>A. niger</i> NRRL3135	NqsSCDTVdQ	GYQCFSETSH	LWGQYAPFFS	LANESVISPE	VPAGCrVTFA
<i>A. fumigatus</i> 13073	GskSCDTVdI	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
<i>A. fumigatus</i> 32722	GskSCDTVdI	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
<i>A. fumigatus</i> 58128	GskSCDTVdI	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
<i>A. fumigatus</i> 26906	GskSCDTVdI	GYQCsPATSH	LWGQYSPFFS	LEDElSVSSK	LPKDCrITLV
<i>A. fumigatus</i> 32239	GskACDTVEI	GYQCsPGTSH	LWGQYSPFFS	LEDElSVSSD	LPKDCrVTFV
<i>E. nidulans</i>	QNHSCNTADG	GYQCFPNVSH	VWGQYSPYFS	IEQESAISeD	VPHGCEVTFV
<i>T. thermophilus</i>	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPQNCKITFV
<i>M. thermophila</i>	ESRPCDTpDI	GFQCgTAISH	FWGQYSPYFS	VpSElDaS..	IPDDCeVTFA
Consensus	NSHSCDTVdG	GYQCFPEISH	LWGQYSPYFS	LEDESAISPD	VPDDC-VTFV
Consensus phytase	NSHSCDTVdG	GYQCFPEISH	LWGQYSPYFS	LEDESAISPD	VPDDCRVTFV
	51				100
<i>A. terreus</i> 9A-1	QVLARHGARs	PThSKtKAYA	AtIAAIQKSA	TaFpGKYAFL	QSYNYSLDSE
<i>A. terreus</i> cbs	QVLARHGARs	PTDSKtKAYA	AtIAAIQKNA	TaLpGKYAFL	KSYNYSMGSE
<i>A. niger</i> var. <i>awamori</i>	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV	TtFDGKYAFL	KTYNYSLGAD
<i>A. niger</i> T213	QVLSRHGARY	PTESKgKkYS	ALIEEIQQNV	TtFDGKYAFL	KTYNYSLGAD
<i>A. niger</i> NRRL3135	QVLSRHGARY	PTDSKgKkYS	ALIEEIQQNA	TtFDGKYAFL	KTYNYSLGAD
<i>A. fumigatus</i> 13073	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> 32722	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> 58128	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> 26906	QVLSRHGARY	PTSSKsKkYK	kLVTAIQaNA	TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> 32239	QVLSRHGARY	PTASKsKkYK	kLVTAIQKNA	TeFKGKFAFL	ETYNITLGAD
<i>E. nidulans</i>	QVLSRHGARY	PTESKsKAYS	GLIEAIQKNA	TsFwGQYAFL	ESYNYTLGAD
<i>T. thermophilus</i>	QLLSRHGARY	PTSSKtELYS	QLISrIQKTA	TaYKGyYAFL	KDYrYqLGAN
<i>M. thermophila</i>	QVLSRHGARA	PTlKRaaSYv	DLlDrIHhGA	IsYgPgYEFL	RTYDYTLGAD
Consensus	QVLSRHGARY	PTSSK-KAYS	ALIEAIQKNA	T-FKGKYAFL	KTYNYTLGAD
Consensus phytase	QVLSRHGARY	PTSSKSKAYS	ALIEAIQKNA	TAFKGKYAFL	KTYNYTLGAD
	101				150
<i>A. terreus</i> 9A-1	ELTPFGrNQL	rDlGaQFYeR	YNALTRhInP	FVRATDASRV	hESAeKFVEG
<i>A. terreus</i> cbs	NLTPFGrNQL	qDlGaQFYRR	YDTLTRhInP	FVRAADSSRV	hESAeKFVEG
<i>A. niger</i> var. <i>awamori</i>	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIIP	FIRSSGSSRV	IASGEKFIEG
<i>A. niger</i> T213	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIIP	FIRSSGSSRV	IASGEKFIEG
<i>A. niger</i> NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESLTRNIVP	FIRSSGSSRV	IASGKKFIEG
<i>A. fumigatus</i> 13073	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
<i>A. fumigatus</i> 32722	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
<i>A. fumigatus</i> 58128	DLTPFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
<i>A. fumigatus</i> 26906	DLTAFGEQQL	VNSGIKFYQR	YKALARSVVP	FIRASGSDRV	IASGEKFIEG
<i>A. fumigatus</i> 32239	DLTPFGEQQM	VNSGIKFYQK	YKALAgSVVP	FIRSSGSDRV	IASGEKFIEG
<i>E. nidulans</i>	DLTiFGENQM	VDSGaKFYRR	YKNLARKnTP	FIRASGSDRV	VASAEKFING
<i>T. thermophilus</i>	DLTPFGENQM	IQlGIKFYnH	YKSLARNaVP	FVRCSGSDRV	IASGrIFIEG
<i>M. thermophila</i>	ELTRtGQQQM	VNSGIKFYRR	YRALARKsIP	FVRTAGqDRV	VhSAENFTQG
Consensus	DLTPFGENQM	VNSGIKFYRR	YKALARK-VP	FVRASGSDRV	IASAEKFIEG
Consensus phytase	DLTPFGENQM	VNSGIKFYRR	YKALARKIVP	FIRASGSDRV	IASAEKFIEG

Fig. 1a

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	151		200
<i>A. terreus</i> 9A-1	FQTARqDDHh	ANpHQPSPrV	DVaIPEGSAY NNTLEHSlCT AFES...STV
<i>A. terreus</i> cbs	FQNARqGDPH	ANpHQPSPrV	DVVIPEGTAY NNTLEHSICT AFEA...STV
<i>A. niger</i> var. <i>awamori</i>	FQSTKLkDPr	AqpgQSSPkI	DVVISEASSs NNTLDPGTCT VFED...SEL
<i>A. niger</i> T213	FQSTKLkDPr	AqpgQSSPkI	DVVISEASSs NNTLDPGTCT VFED...SEL
<i>A. niger</i> NRRL3135	FQSTKLkDPr	AqpgQSSPkI	DVVISEASSs NNTLDPGTCT VFED...SEL
<i>A. fumigatus</i> 13073	FQqAKLADPG	A.TNRAAPAI	SVIIPeSETF NNTLDHGVCT kFEA...SQL
<i>A. fumigatus</i> 32722	FQqAKLADPG	A.TNRAAPAI	SVIIPeSETF NNTLDHGVCT kFEA...SQL
<i>A. fumigatus</i> 58128	FQqAKLADPG	A.TNRAAPAI	SVIIPeSETF NNTLDHGVCT kFEA...SQL
<i>A. fumigatus</i> 26906	FQqAKLADPG	A.TNRAAPAI	SVIIPeSETF NNTLDHGVCT kFEA...SQL
<i>A. fumigatus</i> 32239	FQqANVADPG	A.TNRAAPVI	SVIIPeSETY NNTLDHGVCT kFEA...SQL
<i>E. nidulans</i>	FRKAQLhDHG	S..gQATPVV	NVIIPeIdGF NNTLDHSTCV SFEN...DER
<i>T. thermophilus</i>	FQSAKVlDPh	SDKHDAPPTI	NVIIEEGPSY NNTLDtGSCP VFED...SSg
<i>M. thermophila</i>	FHSALLADRG	STvRPTlPyd	mVVIPETAGa NNTLHNDlCT AFEEgpySTI
Consensus	FQSAKLADPG	S-PHQASPVI	NVIIPeSGSY NNTLDHGTCT AFED---SEL
Consensus phytase	FQSAKLADPG	SQPHQASPVI	DVIIPeSGSY NNTLDHGTCT AFED...SEL
	201		250
<i>A. terreus</i> 9A-1	GDDAvANFTA	VFAPAIaQRL	EADLPgVqLS TDDVvNLMAM CPFETVSlTD
<i>A. terreus</i> cbs	GDAAADNFTA	VFAPAIakRL	EADLPgVqLS ADDVvNLMAM CPFETVSlTD
<i>A. niger</i> var. <i>awamori</i>	ADTVEANFTA	TFAPSIRQRL	ENDLSGVTlT DTEVTyLMDM CSFDTISlSt
<i>A. niger</i> T213	ADTVEANFTA	TFAPSIRQRL	ENDLSGVTlT DTEVTyLMDM CSFDTISlSt
<i>A. niger</i> NRRL3135	ADTVEANFTA	TFVPSIRQRL	ENDLSGVTlT DTEVTyLMDM CSFDTISlSt
<i>A. fumigatus</i> 13073	GDEVAANFTA	lFAPDIRARa	EkHLPgVTLT DEDVVsLMDM CSFDTVARTS
<i>A. fumigatus</i> 32722	GDEVAANFTA	lFAPDIRARa	EkHLPgVTLT DEDVVsLMDM CSFDTVARTS
<i>A. fumigatus</i> 58128	GDEVAANFTA	lFAPDIRARa	EkHLPgVTLT DEDVVsLMDM CSFDTVARTS
<i>A. fumigatus</i> 26906	GDEVAANFTA	lFAPDIRARa	KkHLPgVTLT DEDVVsLMDM CSFDTVARTS
<i>A. fumigatus</i> 32239	GDEVEANFTA	lFAPAIRARI	EkHLPgVqLT DDDVVsLMDM CSFDTVARTA
<i>E. nidulans</i>	ADEiEANFTA	IMGPPIRkRL	ENDLPgIKLT NENViyLMDM CSFDTMARTA
<i>T. thermophilus</i>	GHDAQEKFAK	qFAPAIleKI	KDHLPGVDLA vSDVpyLMDL CPFETLARNh
<i>M. thermophila</i>	GDDAQDtylS	TFAGPitARV	NANLPGANLT DADTVaLMDL CPFETVAssS
Consensus	GDDAEANFTA	TFAPAIRARL	EADLPgVTLT DEDVV-LMDM CPFETVARTS
Consensus phytase	GDDVEANFTA	LFAPAIRARL	EADLPgVTLT DEDVVyLMDM CPFETVARTS
	251		300
<i>A. terreus</i> 9A-1	.....	..DAhTLSPFC	DLFTAtEWtq YNYLlSLDKY YGYGGGNPLG
<i>A. terreus</i> cbs	.....	..DAhTLSPFC	DLFTAaEWtq YNYLlSLDKY YGYGGGNPLG
<i>A. niger</i> var. <i>awamori</i>	.....	..vDTKLSPFC	DLFTHdEWih YDYlQSLkKY YGHGAGNPLG
<i>A. niger</i> T213	.....	..vDTKLSPFC	DLFTHdEWih YDYlRSLkKY YGHGAGNPLG
<i>A. niger</i> NRRL3135	.....	..vDTKLSPFC	DLFTHdEWin YDYlQSLkKY YGHGAGNPLG
<i>A. fumigatus</i> 13073	.....	..DASQLSPFC	QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
<i>A. fumigatus</i> 32722	.....	..DASQLSPFC	QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
<i>A. fumigatus</i> 58128	.....	..DASQLSPFC	QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
<i>A. fumigatus</i> 26906	.....	..DASQLSPFC	QLFTHnEWkk YNYLQSLGKY YGYGAGNPLG
<i>A. fumigatus</i> 32239	.....	..DASELSPFC	AIFTHnEWkk YDYlQSLGKY YGYGAGNPLG
<i>E. nidulans</i>	.....	..HGTELSRFC	AIFTEkEWlq YDYlQSLSKY YGYGAGSPLG
<i>T. thermophilus</i>	.....	..TDT.LSPFC	ALStQeEWqa YDYYQSLGKY YGnGGGNPLG
<i>M. thermophila</i>	sdpataadagg	gNGrPLSPFC	rLFSEsEWra YDYlQSVGKW YGYGPGNPLG
Consensus	-----	-DATELSRFC	ALFTE-EW-- YDYlQSLGKY YGYGAGNPLG
Consensus phytase	.....	..DATELSRFC	ALFTHDEWRQ YDYlQSLGKY YGYGAGNPLG

Fig. 1b

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	301				350
A. terreus 9A-1	PVQGVGWaNE	LMARLTRAPV	HDHTCVNNTL	DASPATFPLN	ATLYADFSHD
A. terreus cbs	PVQGVGWaNE	LIARLTRSPV	HDHTCVNNTL	DANPATFPLN	ATLYADFSHD
A. niger var. awamori	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSNPATFPLN	STLYADFSHD
A. niger T213	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSNPATFPLN	STLYADFSHD
A. niger NRRL3135	PTQGVGYaNE	LIARLTHSPV	HDDTSSNHTL	DSSPATFPLN	STLYADFSHD
A. fumigatus 13073	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 32722	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 58128	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 26906	PAQGIGFtNE	LIARLTRSPV	QDHTSTNsTL	vSNPATFPLN	ATMYVDFSHD
A. fumigatus 32239	PAQGIGFtNE	LIARLTNSPV	QDHTSTNsTL	DSDPATFPLN	ATIYVDFSHD
E. nidulans	PAQGIGFtNE	LIARLTQSPV	QDNTSTNHTL	DSNPATFPLD	rKLYADFSHD
T. thermophilus	PAQGVGFvNE	LIARMTHSPV	QDYTTVNHTL	DSNPATFPLN	ATLYADFSHD
M. thermophila	PTQGVGFvNE	LLARLAgvPV	RDgtTSTNRTL	DGDPrTFPLG	rPLYADFSHD
Consensus	PAQGVGF-NE	LIARLTHSPV	QDHTSTNHTL	DSNPATFPLN	ATLYADFSHD
Consensus phytase	PAQGVGFANE	LIARLTRSPV	QDHTSTNHTL	DSNPATFPLN	ATLYADFSHD
	351				400
A. terreus 9A-1	SNLVSIFWAL	GLYNGTAPLS	qTSVESVSQT	DGYAAAWTVP	FAARAYVEMM
A. terreus cbs	SNLVSIFWAL	GLYNGTkPLS	qTTVEDITrT	DGYAAAWTVP	FAARAYIEMM
A. niger var. awamori	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRIYVEMM
A. niger T213	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRIYVEMM
A. niger NRRL3135	NGIISILFAL	GLYNGTkPLS	TTTVENITQT	DGFSSAWTVP	FASRIYVEMM
A. fumigatus 13073	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFetM
A. fumigatus 32722	NSMVSIFFAL	GLYNGTGPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFetM
A. fumigatus 58128	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFetM
A. fumigatus 26906	NSMVSIFFAL	GLYNGTEPLS	rTSVESaKEl	DGYSASWVVP	FGARAYFetM
A. fumigatus 32239	NGMIPIFFAM	GLYNGTEPLS	qTSeESTKES	NGYSASWAVP	FGARAYFetM
E. nidulans	NSMISIFFAM	GLYNGTQPLS	mDSVESIQEm	DGYAASWTVP	FGARAYFELM
T. thermophilus	NTMTSIFaAL	GLYNGTAKLS	TTEIKSIEET	DGYSAAWTVP	FGGRAYIEMM
M. thermophila	NDMMGVLgAL	GaYDGVPPLD	KTArrDpEEl	GGYAASWAVP	FAARiYVEKM
Consensus	NSMISIFFAL	GLYNGTAPLS	TTSVESIEET	DGYAASWTVP	FGARAYVEMM
Consensus phytase	NSMISIFFAL	GLYNGTAPLS	TTSVESIEET	DGYSASWTVP	FGARAYVEMM
	401				450
A. terreus 9A-1	QC.....	.....RAEKE	PLVRVLVNDR	VMPLHGCPTD	KLGRCKrDAF
A. terreus cbs	QC.....	.....RAEKQ	PLVRVLVNDR	VMPLHGCAVD	NLGRCKrDDF
A. niger var. awamori	QC.....	.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. niger T213	QC.....	.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. niger NRRL3135	QC.....	.....QAEQE	PLVRVLVNDR	VVPLHGCPID	aLGRCTrDSF
A. fumigatus 13073	QC.....	.....KSEKE	PLVRALINDR	VVPLHGCDDVD	KLGRCKLNDF
A. fumigatus 32722	QC.....	.....KSEKE	PLVRALINDR	VVPLHGCDDVD	KLGRCKLNDF
A. fumigatus 58128	QC.....	.....KSEKE	SLVRALINDR	VVPLHGCDDVD	KLGRCKLNDF
A. fumigatus 26906	QC.....	.....KSEKE	PLVRALINDR	VVPLHGCDDVD	KLGRCKLNDF
A. fumigatus 32239	QC.....	.....KSEKE	PLVRALINDR	VVPLHGCDDVD	KLGRCKLNDF
E. nidulans	QC.....	.....E.KKE	PLVRVLVNDR	VVPLHGCAVD	KFGRCTLDDW
T. thermophilus	QC.....	.....DDSDE	PVVRVLVNDR	VVPLHGCEVD	SLGRCKrDDF
M. thermophila	RCsgggggggg	ggegrQEKDE	eMVRVLVNDR	VMTLkGCGAD	ErGMCTLErF
Consensus	QC-----	-----QAEKE	PLVRVLVNDR	VVPLHGCAVD	KLGRCKLDDF
Consensus phytase	QC.....	.....QAEKE	PLVRVLVNDR	VVPLHGCAVD	KLGRCKRDDF

Fig. 1c

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	451		471
A. terreus 9A-1	VAGLSFAQAG	GNWADCF---	~
A. terreus cbs	VEGLSFARAG	NWAECF---	
A. niger var. awamori	VrGLSFARSG	GDWAECsA--	~
A. niger T213	VrGLSFARSG	GDWAECFA--	~
A. niger NRRL3135	VrGLSFARSG	DWAECFA--	
A. fumigatus 13073	VKGLSWARSG	GNWGECFS--	~
A. fumigatus 32722	VKGLSWARSG	GNWGECFS--	~
A. fumigatus 58128	VKGLSWARSG	GNWGECFS--	~
A. fumigatus 26906	VKGLSWARSG	GNWGECFS--	~
A. fumigatus 32239	VKGLSWARSG	NSEQSFS--	
E. nidulans	VEGLNFARSG	GNWkTCFTl~	~
T. thermophilus	VrGLSFARqG	GNWEGCYAas	e
M. thermophila	IESMAFARGN	GKWDlCFA--	~
Consensus	VEGLSFARSG	GNWAECFA--	-
Consensus phytase	VEGLSFARSG	GNWAECFA..	.

Fig. 1d

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CP-1  
Eco RI M G V F V V L L S I A T L F G S T  
TATATGAATTCATGGGCGTGTTCGTGCTACTGTCCATTGCCACCTTGTTTCGGTTCCA  
1 -----+-----+-----+-----+-----+-----+ 60  
ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAAACAAGCCAAGGT  
  
S G T A L G P R G N S H S C D T V D G G  
CATCCGGTACCGCCTTGGGTCTCGTGTAATTCTCACTCTTGTGACACTGTTGACGGTG  
61 -----+-----+-----+-----+-----+-----+ 120  
GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC  
CP-2  
CP-3  
Y Q C F P E I S H L W G Q Y S P Y F S L  
GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT  
121 -----+-----+-----+-----+-----+-----+ 180  
CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTATGAAGAGAA  
  
E D E S A I S P D V P D D C R V T F V Q  
TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTT  
181 -----+-----+-----+-----+-----+-----+ 240  
ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG  
CP-4  
CP-5  
V L S R H G A R Y P T S S K S K A Y S A  
AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTTCTTCTAAGTCTAAGGCTTACTCTG  
241 -----+-----+-----+-----+-----+-----+ 300  
TTCAAAACAGATCTGTGCCACGATCTATGGGTTGAAGAAGATTCAGATTCCGAATGAGAC  
  
L I E A I Q K N A T A F K G K Y A F L K  
CTTTGATTGAAGCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA  
301 -----+-----+-----+-----+-----+-----+ 360  
GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT  
CP-6  
CP-7  
T Y N Y T L G A D D L T P F G E N Q M V  
AGACTTACAACCTACACTTTGGGTGCTGACGACTTGACTCCATTTCGGTGAAAACCAAATGG  
361 -----+-----+-----+-----+-----+-----+ 420  
TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC  
  
N S G I K F Y R R Y K A L A R K I V P F  
TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT  
421 -----+-----+-----+-----+-----+-----+ 480  
AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA  
CP-8  
CP-9  
I R A S G S D R V I A S A E K F I E G F  
TCATTAGAGCTTCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT  
481 -----+-----+-----+-----+-----+-----+ 540  
AGTAATCTCGAAGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA  
  
Q S A K L A D P G S Q P H Q A S P V I D  
TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG  
541 -----+-----+-----+-----+-----+-----+ 600  
AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTTCAAGAGGTCAATAAC

Fig. 2a

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CP-10

CP-11

V I I P E G S G Y N N T L D H G T C T A  
ACGTTATTATTCCAGAAGGaTCcGGTTACAACAACACTTTGGACCACGGTACTTGTACTG  
601 -----+-----+-----+-----+-----+-----+ 660  
TGCAATAATAAGGTCTTCcTAGgCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGAC

F E D S E L G D D V E A N F T A L F A P  
CTTTCGAAGACTCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTTCGCTC  
661 -----+-----+-----+-----+-----+-----+ 720  
GAAAGCTTCTGAGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAG

CP-12

A I R A R L E A D L P G V T L T D E D V  
CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACG  
721 -----+-----+-----+-----+-----+-----+ 780  
GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGCTTCTGC

CP-13

V Y L M D M C P F E T V A R T S D A T E  
TTGTTTACTTGATGGACATGTGTCCATTTCGAAACTGTTGCTAGAACTTCTGACGCTACTG  
781 -----+-----+-----+-----+-----+-----+ 840  
AACAAATGAACTACCTGTACACAGGTAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

L S P F C A L F T H D E W R Q Y D Y L Q  
AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGACAATACGACTACTTGC  
841 -----+-----+-----+-----+-----+-----+ 900  
TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTGTTATGCTGATGAACG

CP-14

CP-15

S L G K Y Y G Y G A G N P L G P A Q G V  
AATCTTTGGGTAAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTG  
901 -----+-----+-----+-----+-----+-----+ 960  
TTAGAAACCCATTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTGAGTTCCAC

G F A N E L I A R L T R S P V Q D H T S  
TTGGTTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT  
961 -----+-----+-----+-----+-----+-----+ 1020  
AACCAAAGCGATTGCTTAACCTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA

CP-16

CP-17

T N H T L D S N P A T F P L N A T L Y A  
CTACTAACCACACTTTGGACTCTAACCAGCTACTTTCCCATTTGAACGCTACTTTGTACG  
1021 -----+-----+-----+-----+-----+-----+ 1080  
GATGATTGGTGTGAAACCTGAGATTGGGTGATGAAAGGGTAACTTGCGATGAAACATGC

D F S H D N S M I S I F F A L G L Y N G  
CTGACTTCTCTCACGACAACTCTATGATTTCTATTTTCTTCGCTTTGGGTTTGTACAACG  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GACTGAAGAGAGTGCTGTTGAGATACTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC

CP-18

CP-19

T A P L S T T S V E S I E E T D G Y S A  
GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTG  
1141 -----+-----+-----+-----+-----+-----+ 1200  
CATGACGAGGTAACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGAC

**Fig. 2b**

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S W T V P F G A R A Y V E M M Q C Q A E  
CTTCTTGGACTGTTCCATTTCGGTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTG  
1201 -----+-----+-----+-----+-----+-----+ 1260  
GAAGAACCTGACAAGGTAAGCCACGATCTCGAATGCAACTTTACTACGTTACAGTTCGAC  
CP-20  
CP-21  
K E P L V R V L V N D R V V P L H G C A  
AAAAGGAACCATTTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTG  
1261 -----+-----+-----+-----+-----+-----+ 1320  
TTTTCCTTGGTAACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACAC  
V D K L G R C K R D D F V E G L S F A R  
CTGTTGACAAGTTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTA  
1321 -----+-----+-----+-----+-----+-----+ 1380  
GACAACTGTTCAACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGAT  
CP-22  
S G G N W A E C F A \* Eco RI  
GATCTGGTGGTAACTGGGCTGAATGTTTCGCTTAAGAATTCATATA  
1381 -----+-----+-----+-----+-----+-----+ 1426  
CTAGACCACCATTGACCCGACTTACAAAGCGAATTCTTAAGTATAT

Fig. 2c

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		1				50
<i>P. involutus</i>	(phyA1)	SvP.KnTAPt	FPIPeseQrn	WSPYSPYFPL	AeYkAPPAGC	QInQVNIIQR
<i>P. involutus</i>	(phyA2)	SvP.RniAPK	FSIPeseQrn	WSPYSPYFPL	AeYkAPPAGC	EInQVNIIQR
<i>T. pubescens</i>		hiPlRdTSAc	LdVTrDvQqs	WSmYSPYFPA	AtYvAPPASC	QInQVHIIQR
<i>A. pediades</i>		GgvvQaTfvQ	pfFPpQiQds	WAAYTPYYPV	qaYtPPPkDC	KItQVNIIQR
<i>P. lycii</i>		StQfsfvAAQ	LPIPaQntsn	WGPYdPFFPV	EpYaAPPEGC	tVtQVNLIQR
<b>Basidio</b>		<b>S-P-R-TAAQ</b>	<b>LPiP-Q-Q--</b>	<b>WSPYSPYFPV</b>	<b>A-Y-APPAGC</b>	<b>QI-QVNIIQR</b>
		51				100
<i>P. involutus</i>	(phyA1)	HGARFPTSGA	TTRIKAGLTK	LQGvqnftDA	KFNFIksfky	dLGnsDLVPF
<i>P. involutus</i>	(phyA2)	HGARFPTSGA	ATRIKAGLSK	LQSvqnftDP	KFDfIksfTY	dLGtsDLVPF
<i>T. pubescens</i>		HGARFPTSGA	AkRIQTAVAK	LKAAsnyTDP	lLAFVtNyTY	sLGqDsLVeL
<i>A. pediades</i>		HGARFPTSGA	GTRIQAAVKK	LQSAktyTDP	RLDFLtNyTY	tLGhDDLVPF
<i>P. lycii</i>		HGARWPTSGA	rSRqvAAVAK	IQmArpfTDP	KYEFLnDfvY	kFGvADLLPF
<b>Basidio</b>		<b>HGARFPTSGA</b>	<b>ATRIQAAVAK</b>	<b>LQSA---TDP</b>	<b>KLDFL-N-TY</b>	<b>-LG-DDLVPF</b>
		101				150
<i>P. involutus</i>	(phyA1)	GAAQSfDAGQ	EAFARYSkLV	SkNNLPFIRA	dGSDRVVDSA	TNWTAGFAsA
<i>P. involutus</i>	(phyA2)	GAAQSfDAGl	EvFARYSkLV	SsDNLPFIRS	dGSDRVVDTA	TNWTAGFAsA
<i>T. pubescens</i>		GATQSSEAGQ	EAFTRYSSLV	SaDELPFVRA	SGSDRVVATA	nNWTAGFALA
<i>A. pediades</i>		GALQSSQAGE	ETFqRYSfLV	SkENLPFVRA	SSSNRVVDSA	TNWTEGFSaA
<i>P. lycii</i>		GAnQShQTGt	DmYTRYStLf	egGDVPFVRA	AGdQRVVDSS	TNWTAGFGdA
<b>Basidio</b>		<b>GA-QSSQAGQ</b>	<b>EAFTRYs-LV</b>	<b>S-DNLPFVRA</b>	<b>SGSDRVVDSA</b>	<b>TNWTAGFA-A</b>
		151				200
<i>P. involutus</i>	(phyA1)	ShNTvqPkLn	LILPQtGNDT	LEDNMCPaAG	DSDPQvNaWL	AVafPSITAR
<i>P. involutus</i>	(phyA2)	SrNAiqPkLd	LILPQtGNDT	LEDNMCPaAG	ESDPQvDaWL	AsafPSVTAQ
<i>T. pubescens</i>		SsNSitPvLs	VIISEaGNDT	LDDNMCPaAG	DSDPQvNqWL	AqFAPPMTAR
<i>A. pediades</i>		ShHvlnPiLf	VILSEslNDT	LDDaMCPnAG	sSDPQtGiWt	SIYGTPIAnR
<i>P. lycii</i>		SgETvlPtLq	VVLqEeGNcT	LcNNMCPnEv	DGDest.tWL	GVFAPnITAR
<b>Basidio</b>		<b>S-NT--P-L-</b>	<b>VILSE-GNDT</b>	<b>LDDNMCP-AG</b>	<b>DSDPQ-N-WL</b>	<b>AVFAPPITAR</b>
		201				250
<i>P. involutus</i>	(phyA1)	LNAAAPSVNL	TDtDAfNLvs	LCAFlTVSke	kkSdFCtLFE	giPGsFeAFa
<i>P. involutus</i>	(phyA2)	LNAAAPGANL	TDaDAfNLvs	LCPFmTVSke	qkSdFCtLFE	giPGsFeAFa
<i>T. pubescens</i>		LNAGAPGANL	TDtDTyNLlt	LCPFETVAtE	rrSeFCDIYE	elQAE.dAFa
<i>A. pediades</i>		LNqqAPGANI	TAAvvsNLip	LCAFETIvke	tpSpFCNLf.	.tPEEFaqFe
<i>P. lycii</i>		LNAAAPSANL	SDsDAItLmd	MCPFDTLSSg	naSpFCDLf.	.tAEEYvSYe
<b>Basidio</b>		<b>LNAAAPGANL</b>	<b>TD-DA-NL--</b>	<b>LCPFETVS-E</b>	<b>--S-FCDLFE</b>	<b>--PEEF-AF-</b>

Fig. 3a

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	251		300
<i>P. involutus</i> (phyA1)	YgGDLDKfYg	TGYGQeLGPV	QGVGYVNELI ARLTnsAVRD NTQTNRTLDA
<i>P. involutus</i> (phyA2)	YaGDLDKfYg	TGYGQALGPV	QGVGYINELL ARLTnsAVnD NTQTNRTLDA
<i>T. pubescens</i>	YnADLDKfYg	TGYGQPLGPV	QGVGYINELI ARLTaQnVsD HTQTNsTLDS
<i>A. pediades</i>	YfGDLDKfYg	TGYGQPLGPV	QGVGYINELL ARLTempVRD NTQTNRTLDS
<i>P. lycii</i>	YyyDLdkYyG	TGpGNALGPV	QGVGYVNELL ARLTgQAVRD ETQTNRTLDS

**Basidio** Y-GDLDKfYg TGYGQPLGPV QGVGYINELL ARLT-QAVRD NTQTNRTLDS

	301		350
<i>P. involutus</i> (phyA1)	SPvTFPLNKT	FYADFSHDNl	MVAVFSAMGL FrQPAPLsTS vPNPwRTWrT
<i>P. involutus</i> (phyA2)	APdTFPLNKT	MYADFSHDNl	MVAVFSAMGL FrQSAPLsTS tPDPNRTWLT
<i>T. pubescens</i>	SPeTFPLNRT	LYADFSHDNQ	MVAIFSAMGL FNQSAPLDPT tPDPaRTFLv
<i>A. pediades</i>	SPlTFPLDRS	IYADLSHDNQ	MIAIFSAMGL FNQSSPLDPS fPNPKRTWVT
<i>P. lycii</i>	dPaTFPLNRT	FYADFSHDNt	MVPIFAALGL FNaTA.LDP1 kPDeNRlWVd

**Basidio** SP-TFPLNRT FYADFSHDNQ MVAIFSAMGL FNQSAPLDPS -PDPNRTWVT

	351		400
<i>P. involutus</i> (phyA1)	SsLVPFSGRM	VVERLsC..f	GT.....tkV RVLVQDqVQP
<i>P. involutus</i> (phyA2)	SsVVPFSARM	aVERLsC..a	GT.....tkV RVLVQDqVQP
<i>T. pubescens</i>	kKIVPFSGRM	VVERLdC..g	GA.....qsV RLLVNDAVQP
<i>A. pediades</i>	SRLtPFSGRM	VtERLlCqrd	GTgsggpsri mrngnvqtfv RILVNDAVQP
<i>P. lycii</i>	SKLVPFSGHM	tVEKLaC...	.....sgkeaV RVLVNDAVQP

**Basidio** SKLVPFSGRM VVERL-C--- GT-----V RVLVNDAVQP

	401		441
<i>P. involutus</i> (phyA1)	LEFCGGDrNG	lCTLakFVES	QtFARsDGaG DFEKCFATSa ~
<i>P. involutus</i> (phyA2)	LEFCGGDqDG	lCALDkFVES	QaYARsGGaG DFEKCLATTv ~
<i>T. pubescens</i>	LAFCGADtsG	vCTLDaFVES	QaYARNDGEG DFEKCFAT~~ ~
<i>A. pediades</i>	LKFCGGDmDS	lCTLEaFVES	QkYAREdGQG DFEKCFD~~~ ~
<i>P. lycii</i>	LEFCGG.vDG	vCeLsAFVES	QtYARENGQG DfAKCgfvPs e

**Basidio** LEFCGGD-DG -CTLDaFVES Q-YAREdGQG DFEKCFATP- -

Fig. 3b

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	1				50
<i>A. terreus</i> 9a1	KhSDCNSVDh	GYQCfPELSH	kWGLYAPYFS	LqDESPFP1D	VPeDCHITFV
<i>A. terreus</i> cbs	NhsdCtSVDr	GYQCfPELSH	kWGLYAPYFS	LqDESPFP1D	VPdDCHITFV
<i>A. niger</i> var. <i>awamori</i>	NqSTCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESAISPD	VPaGCRVTFa
<i>A. niger</i> NRRL3135	NqsSCDTVDq	GYQCfSEtSH	LWGQYAPFFS	LANESvISPE	VPaGCRVTFa
<i>A. fumigatus</i> 13073	GskSCDTVDl	GYQCSPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
<i>A. fumigatus</i> 32722	GskSCDTVDl	GYQCSPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
<i>A. fumigatus</i> 58128	GskSCDTVDl	GYQCSPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
<i>A. fumigatus</i> 26906	GskSCDTVDl	GYQCSPAtSH	LWGQYSPFFS	LEDElSVSSK	LPkDCRITLV
<i>A. fumigatus</i> 32239	GskACDTVEl	GYQCSPGtSH	LWGQYSPFFS	LEDElSVSSD	LPkDCRVTFV
<i>E. nidulans</i>	QNHSCNTaDG	GYQCfPNVSH	VWGQYSPYFS	IEQESAISeD	VPhGCeVTFV
<i>T. thermophilus</i>	DSHSCNTVEG	GYQCrPEISH	sWGQYSPFFS	LADQSEISPD	VPqNCKITFV
<i>T. lanuginosus</i>	~~~~~	~~~~~nvDIAR	hWGQYSPFFS	LAEvSEISPA	VPkGCRVeFV
<i>M. thermophila</i>	ESRPCDTpDl	GFQCgTAISH	FWGQYSPYFS	VPsElDaS..	IPdDCeVTFa
Basidio	xSxPxrxTAA	qLPipxQxqx	xWSPYSPYFP	VaxyxA....	pPaGCQIxqV
Consensus	NSHSCDTVDG	GYQC-PEISH	LWGQYSPFFS	LADESAISPD	VP-GCRVTfV
Fcp10	NSHSCDTVDG	GYQCFPEISH	LWGQYSPFFS	LADESAISPD	VPKGCRVTfV
	51				100
<i>A. terreus</i> 9a1	QVLARHGARs	PTThSKTKaYA	AtIaAIQKSA	TaFpGKYAFL	QSYNYSLDSE
<i>A. terreus</i> cbs	QVLARHGARs	PTdSKTKaYA	AtIaAIQKNA	TaLpGKYAFL	KSYNYSMGSE
<i>A. niger</i> var. <i>awamori</i>	QVLSRHGARY	PTeSKGKKYS	ALIEEIQQNv	TtFDGKYAFL	KTYNYSLGAD
<i>A. niger</i> NRRL3135	QVLSRHGARY	PTdSKGKKYS	ALIEEIQQNA	TtFDGKYAFL	KTYNYSLGAD
<i>A. fumigatus</i> 13073	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> 32722	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> 58128	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> 26906	QVLSRHGARY	PTSSKSKKYk	kLVtAIQaNA	TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> 32239	QVLSRHGARY	PTASKSKKYk	kLVtAIQKNA	TeFKGKFAFL	ETNYNYTLGAD
<i>E. nidulans</i>	QVLSRHGARY	PTeSKSKaYS	GLIEAIQKNA	TsFwGQYAFL	ESYNYTLGAD
<i>T. thermophilus</i>	QLLSRHGARY	PTSSKTELYS	qLIsrIQKtA	TaYKGyYAFL	KdYrYqLGAN
<i>T. lanuginosus</i>	QVLSRHGARY	PTAhKSEvYA	ELLqrIQDtA	TeFKGDFAFL	RdYaYhLGAD
<i>M. thermophila</i>	QVLSRHGARA	PTlkRAAsYv	DLIdrIHhGA	isYgPgYEFL	RTYDYTLGAD
Basidio	NIIqRHGARF	PTSGaAtRiq	AaVakLQsax	xxTDPKLDFL	xxxtYxLGxD
Consensus	QVLSRHGARY	PTSSKSKKYs	ALI-AIQKNA	T-FKGKYAFL	KTYNYTLGAD
Fcp10	QVLSRHGARY	PTSSKSKKYs	ALIEAIQKNA	TAFKGKYAFL	KTYNYTLGAD
	101				150
<i>A. terreus</i> 9a1	ELTPFGrNQL	rDlGaQFYeR	YNAL.TRhIn	PFVRATDAsR	VhESAeKFVE
<i>A. terreus</i> cbs	NLTPFGrNQL	qDlGaQFYRR	YDTL.TRhIn	PFVRAADSsR	VhESAeKFVE
<i>A. niger</i> var. <i>awamori</i>	DLTPFGEQEL	VNSGIKFYQR	YESL.TRnII	PFIRSSGSsR	VIASGEKFIE
<i>A. niger</i> NRRL3135	DLTPFGEQEL	VNSGIKFYQR	YESL.TRnIV	PFIRSSGSsR	VIASGKKFIE
<i>A. fumigatus</i> 13073	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
<i>A. fumigatus</i> 32722	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
<i>A. fumigatus</i> 58128	DLTPFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
<i>A. fumigatus</i> 26906	DLTAFGEQQL	VNSGIKFYQR	YKAL.ARsVV	PFIRASGSDR	VIASGEKFIE
<i>A. fumigatus</i> 32239	DLTPFGEQQM	VNSGIKFYQK	YKAL.AgsVV	PFIRSSGSDR	VIASGEKFIE
<i>E. nidulans</i>	DLTiFGENQM	VDSGaKFYRR	YKnL.ARknt	PFIRASGSDR	VVASAEKFIn
<i>T. thermophilus</i>	DLTPFGENQM	IQlGIKFYnH	YKSL.ARnaV	PFVRCSGSDR	VIASGrIFIE
<i>T. lanuginosus</i>	NLTRFGEEQM	MESGrQFYHR	YREq.AReIV	PFVRAAGSAR	VIASAEfFnR
<i>M. thermophila</i>	ELTRtGQQQM	VNSGIKFYRR	YRAL.ARksI	PFVRTAGqDR	VVhSAENFtQ
Basidio	DLvPFGAxQs	sQAGqEaFtR	YsxLvSxdnL	PFVRASGSDR	VVDSAtNwtA
Consensus	DLTPFGEQQM	VNSGIKFYRR	YKAL-AR-IV	PFVRASGSDR	VIASAEKFIE
Fcp10	DLTPFGEQQM	VNSGIKFYRR	YKAL.ARkIV	PFVRASGSDR	VIASAEKFIE

Fig. 4a

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	151				200
<i>A. terreus</i> 9a1	GFQTARqDDh	hAnphQPSPr	VDVaIPEGsA	YNNTLEHSLC	TAFes...St
<i>A. terreus</i> cbs	GFQNARqGDP	hAnphQPSPr	VDVVIPEGtA	YNNTLEHSIC	TAFea...St
<i>A. niger</i> var. <i>awamori</i>	GFQSTKLkDP	rAqpgQSSPk	IDVVISeAsS	sNNTLDpGtC	TvFed...SE
<i>A. niger</i> NRRL3135	GFQSTKLkDP	rAqpgQSSPk	IDVVISeAsS	sNNTLDpGtC	TvFed...SE
<i>A. fumigatus</i> 13073	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEa...SQ
<i>A. fumigatus</i> 32722	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEa...SQ
<i>A. fumigatus</i> 58128	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEa...SQ
<i>A. fumigatus</i> 26906	GFQqAKLADP	gAt.nRAAPa	ISVIIPESeT	FNNTLDHGVC	TkFEa...SQ
<i>A. fumigatus</i> 32239	GFQqANVADP	gAt.nRAAPV	ISVIIPESeT	YNNTLDHSVC	TnFEa...SE
<i>E. nidulans</i>	GFRkAQLhDh	g.s.gQATPV	VNVIIEIdG	FNNTLDHStC	vSFEn...dE
<i>T. thermophilus</i>	GFQSAKVlDP	hSdKhDAPpt	INVIIeEGpS	YNNTLDtGsC	PvFed...Ss
<i>T. lanuginosus</i>	GFQdAKdrDP	rSnkdQAePV	INVIIEEtG	sNNTLDgltC	PAaEe...Ap
<i>M. thermophila</i>	GFHSALLADR	gStvrPTlPy	dmVVIPETaG	aNNTLHNDLC	TAFEegPySt
Basidio	GFaxA.....	..sxntxxPx	LxVILSExg.	.NDTLDDNMC	.....PxAG
Consensus	GFQSAKLADP	-A---QASPV	INVIIPEG-G	YNNTLDHGLC	TAFE--P-SE
Fcp10	GFQSAKLADP	GANPHQASPV	INVIIPEGAG	YNNTLDHGLC	TAFEE...SE
	201				250
<i>A. terreus</i> 9a1	VGDDavANFT	AVFAPAIaQr	LEAdLPGVQL	StDDVVNLMA	MCPFETVSlT
<i>A. terreus</i> cbs	VGDAaADNFT	AVFAPAIaKr	LEAdLPGVQL	SADDVVNLMA	MCPFETVSlT
<i>A. niger</i> var. <i>awamori</i>	LADtVEANFT	AtFAPSIRqR	LEndLSGvtL	TDtEVtyLMD	MCSFDTISSt
<i>A. niger</i> NRRL3135	LADtVEANFT	AtFvPSIRqR	LEndLSGvtL	TDtEVtyLMD	MCSFDTISSt
<i>A. fumigatus</i> 13073	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL	TDEDVVSLMD	MCSFDTVArT
<i>A. fumigatus</i> 32722	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL	TDEDVVSLMD	MCSFDTVArT
<i>A. fumigatus</i> 58128	LGDEVAANFT	ALFAPdIRAR	aEkhlPGVtL	TDEDVVSLMD	MCSFDTVArT
<i>A. fumigatus</i> 26906	LGDEVAANFT	ALFAPdIRAR	aKkhLPGVtL	TDEDVVSLMD	MCSFDTVArT
<i>A. fumigatus</i> 32239	LGDEVEANFT	ALFAPAIRAR	IEkhLPGVQL	TDDDVVSLMD	MCSFDTVArT
<i>E. nidulans</i>	rADEIEANFT	AIMGPPIRkR	LEndLPGIKL	TNENViYlMD	MCSFDTMArT
<i>T. thermophilus</i>	gGHDAQEKFA	kqFAPAILEK	IKDhLPGVDL	AvsDVpyLMD	LCPFETLArN
<i>T. lanuginosus</i>	.DptqpAEFl	qVFGPRVlkK	ItkhMPGVNL	TLEDVplFMD	LCPFDTVGsd
<i>M. thermophila</i>	IGDDaQDtYl	StFAGPITAR	VNAnLPGaNL	TDADtVaLMD	LCPFETVAss
Basidio	dSDpqqnxWl	AVFAPPITAR	LNAAaPGaNL	TDxDaxNLxx	LCPFETVS..
Consensus	LGDDVEANFT	AVFAPPIRAR	LEA-LPGVNL	TDEDVVNLMD	MCPFDTVA-T
Fcp10	LGDDVEANFT	AVFAPPIRAR	LEAHLPGVNL	TDEDVVNLMD	MCPFDTVART
	251				300
<i>A. terreus</i> 9a1	dD..Aht...	.....LSPF	CDLFTa..tE	WtQYNYLlSL	dKYYGYGGGN
<i>A. terreus</i> cbs	dD..Aht...	.....LSPF	CDLFTa..aE	WtQYNYLlSL	dKYYGYGGGN
<i>A. niger</i> var. <i>awamori</i>	Tv..DTK...	.....LSPF	CDLFTH..dE	WiHYDYLQSL	kKYYGHGAGN
<i>A. niger</i> NRRL3135	Tv..DTK...	.....LSPF	CDLFTH..dE	WiNYDYLQSL	kKYYGHGAGN
<i>A. fumigatus</i> 13073	SD..ASQ...	.....LSPF	CQLFTH..nE	WkKYNLYQSL	gKYYGYGAGN
<i>A. fumigatus</i> 32722	SD..ASQ...	.....LSPF	CQLFTH..nE	WkKYNLYQSL	gKYYGYGAGN
<i>A. fumigatus</i> 58128	SD..ASQ...	.....LSPF	CQLFTH..nE	WkKYNLYQSL	gKYYGYGAGN
<i>A. fumigatus</i> 26906	SD..ASQ...	.....LSPF	CQLFTH..nE	WkKYNLYQSL	gKYYGYGAGN
<i>A. fumigatus</i> 32239	AD..ASE...	.....LSPF	CAIFTH..nE	WkKYDYlQSL	gKYYGYGAGN
<i>E. nidulans</i>	AH..GTE...	.....LSPF	CAIFTE..kE	WlQYDYlQSL	sKYYGYGAGS
<i>T. thermophilus</i>	ht..DT....	.....LSPF	CALStQ..eE	WqaYDYlQSL	gKYYGnGGGN
<i>T. lanuginosus</i>	PvlfPrQ...	.....LSPF	CHLFTa..dD	WmaYDYlYTL	dKYYSHGGGS
<i>M. thermophila</i>	SsdpaTadag	ggngRpLSPF	CrLFSE..sE	WraYDYlQSV	gKWYGYGPgN
Basidio	.....	...xexxSxF	CDLFexxpeE	FxaFxYxgdL	dKFYGTgYGO
Consensus	SD--ATQ---	-----LSPF	CDLFTH---E	W-QYDYlQSL	-KYYGYGAGN
Fcp10	SD..ATQ...	.....LSPF	CDLFTH..DE	WIQYDYlQSL	GKYYGYGAGN

Fig. 4b

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	301		350
<i>A. terreus</i> 9a1	PLGPvQGVGW aNELMARLTR	<i>A. PVHDHTCv</i>	NNTLDASPAT FPLNATLYAD
<i>A. terreus</i> cbs	PLGPvQGVGW aNELIARLTR	<i>S. PVHDHTCv</i>	NNTLDANPAT FPLNATLYAD
<i>A. niger</i> var. <i>awamori</i>	PLGPTQGVGY aNELIARLTH	<i>S. PVHDDTSS</i>	NHTLDSNPAT FPLNSTLYAD
<i>A. niger</i> NRRL3135	PLGPTQGVGY aNELIARLTH	<i>S. PVHDDTSS</i>	NHTLDSSPAT FPLNSTLYAD
<i>A. fumigatus</i> 13073	PLGPAQGIGF tNELIARLTR	<i>S. PVQDHTST</i>	NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> 32722	PLGPAQGIGF tNELIARLTR	<i>S. PVQDHTST</i>	NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> 58128	PLGPAQGIGF tNELIARLTR	<i>S. PVQDHTST</i>	NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> 26906	PLGPAQGIGF tNELIARLTR	<i>S. PVQDHTST</i>	NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> 32239	PLGPAQGIGF tNELIARLTN	<i>S. PVQDHTST</i>	NsTLvSNPAT FPLNATMYvD
<i>E. nidulans</i>	PLGPAQGIGF tNELIARLTQ	<i>S. PVQDHTST</i>	NHTLDSNPAT FPLDrkLYAD
<i>T. thermophilus</i>	PLGPAQGVGF vNELIARMTH	<i>S. PVQDYTTv</i>	NHTLDSNPAT FPLNATLYAD
<i>T. lanuginosus</i>	AFGPSRGVGF vNELIARMTg	<i>NlPVKDHTTv</i>	NHTLDdNPET FPLDAvLYAD
<i>M. thermophila</i>	PLGPTQGVGF vNELLARLA.	<i>GvPVRDgTST</i>	NRTLdGDPRT FPLGrPLYAD
Basidio	PLGPvQGVGY iNELLARLTx	<i>qa.VRDNTqT</i>	NRTLdSSPxT FPLNrTFYAD
Consensus	PLGPAQGVGF -NELIARLTH	<i>S-PVQDHTST</i>	NHTLDSNPAT FPLNATLYAD
Fcp10	PLGPAQGVGF VNELIARLTH	<i>S. PVQDHTST</i>	NHTLDSNPAT FPLNATLYAD
	351		400
<i>A. terreus</i> 9a1	FSHDSnLVSI FWALGLYNGT	aPLSqtSVE.	.SvsQTDGYA AAWTVPFAAR
<i>A. terreus</i> cbs	FSHDSnLVSI FWALGLYNGT	kPLSqtTVE.	.ditrTDGYA AAWTVPFAAR
<i>A. niger</i> var. <i>awamori</i>	FSHDNGIISI LFALGLYNGT	kPLSTTTVE.	.NitQTDGFS SAWTVPFAAR
<i>A. niger</i> NRRL3135	FSHDNGIISI LFALGLYNGT	kPLSTTTVE.	.NitQTDGFS SAWTVPFAAR
<i>A. fumigatus</i> 13073	FSHDNSMVISI FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS ASWvVPFGAR
<i>A. fumigatus</i> 32722	FSHDNSMVISI FFALGLYNGT	gPLSrTSVE.	.SaKElDGYS ASWvVPFGAR
<i>A. fumigatus</i> 58128	FSHDNSMVISI FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS ASWvVPFGAR
<i>A. fumigatus</i> 26906	FSHDNSMVISI FFALGLYNGT	ePLSrTSVE.	.SaKElDGYS ASWvVPFGAR
<i>A. fumigatus</i> 32239	FSHDNGMIPI FFAMGLYNGT	ePLSqtSee.	.StKESNGYS ASWAVPFGAR
<i>E. nidulans</i>	FSHDNSMISI FFAMGLYNGT	qPLSmdSVE.	.SiQEmDGYA ASWTVPFGAR
<i>T. thermophilus</i>	FSHDNTMtSI FaALGLYNGT	akLSTTeIK.	.SiEETDGYS AAWTVPFGGR
<i>T. lanuginosus</i>	FSHDNTMtGI FsAMGLYNGT	kPLSTskiQP	pTgAAADGYA ASWTVPFAAR
<i>M. thermophila</i>	FSHDNqMMGV LgALGaYDgV	pPLdkTA..R	rdpEElGGYA ASWAVPFAAR
Basidio	FSHDNqMVAI FsAMGLFNqS	aPLdPSxpDP	nrt.....Wv TSk1VPFSAR
Consensus	FSHDNTMVISI FFALGLYNGT	-PLSTTSVEP	-S-EETDGYS ASWTVPFAAR
Fcp10	FSHDNTMVISI FFALGLYNGT	KPLSTTSVE.	.SIEETDGYS ASWTVPFAAR
	401		450
<i>A. terreus</i> 9a1	AYVEMMQC.. ra.....	.....EKEPL	VRVLVNDVRM PLHGCPtDKL
<i>A. terreus</i> cbs	AYIEMMQC.. ra.....	.....EKQPL	VRVLVNDVRM PLHGCAVDNL
<i>A. niger</i> var. <i>awamori</i>	lyVEMMQC.. Qa.....	.....EQEPL	VRVLVNDRVV PLHGCPIDaL
<i>A. niger</i> NRRL3135	lyVEMMQC.. Qa.....	.....EQEPL	VRVLVNDRVV PLHGCPVDaL
<i>A. fumigatus</i> 13073	AYfEtMQC.. Ks.....	.....EKEPL	VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 32722	AYfEtMQC.. Ks.....	.....EKEPL	VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 58128	AYfEtMQC.. Ks.....	.....EKESL	VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 26906	AYfEtMQC.. Ks.....	.....EKEPL	VRaLINDRVV PLHGCDVDKL
<i>A. fumigatus</i> 32239	AYfEtMQC.. Ks.....	.....EKEPL	VRaLINDRVV PLHGCAVDKL
<i>E. nidulans</i>	AYfELMQC.. E.....	.....KKEPL	VRVLVNDRVV PLHGCAVDKF
<i>T. thermophilus</i>	AYIEMMQC.. Dd.....	.....sDEPV	VRVLVNDRVV PLHGCEVDSL
<i>T. lanuginosus</i>	AYVELLRC.. Etetsseeee	EG...EDEPF	VRVLVNDRVV PLHGCrVDRW
<i>M. thermophila</i>	iyVEkMRC.. sgggggggggg	EGrqeKDEeM	VRVLVNDVRM TLkGCGaDer
Basidio	mvVerLxCxx xgtxxxxxxxx	xxxxxxxxxxx	VRVLVNDaVq PLEfCGgDxd
Consensus	AYVEMMQC-- E-----	EG---EKEPL	VRVLVNDRVV PLHGCGVDKL
Fcp10	AYVEMMQC.. EA.....	.....EKEPL	VRVLVNDRVV PLHGCGVDKL

Fig. 4c

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	451		482
A. terreus 9a1	GRCKrDAFVA	GLSFAQAG..	GNWADCF--- --
A. terreus cbs	GRCKrDDFVE	GLSFARAG..	GNWAECF--- --
A. niger var. awamori	GRCtrDsFVr	GLSFARSG..	GDWAECsA-- --
A. niger NRRL3135	GRCtrDsFVr	GLSFARSG..	GDWAECFA-- --
A. fumigatus 13073	GRCKlNDFVK	GLSWARSG..	GNWGECFS-- --
A. fumigatus 32722	GRCKlNDFVK	GLSWARSG..	GNWGECFS-- --
A. fumigatus 58128	GRCKlNDFVK	GLSWARSG..	GNWGECFS-- --
A. fumigatus 26906	GRCKlNDFVK	GLSWARSG..	GNWGECFS-- --
A. fumigatus 32239	GRCKlKDFVK	GLSWARSG..	GNSEQSFS-- --
E. nidulans	GRCtlDDWVE	GLNFARSG..	GNWKtCFTl- --
T. thermophilus	GRCKrDDFVr	GLSFARqG..	GNWEGCYAas e-
T. lanuginosus	GRCRrDEWIK	GLTFARqG..	GHWDrCF--- --
M. thermophila	GmCtlErFIE	SMAFARGN..	GKWDlCFA-- --
Basidio	GxCtlDAFVE	SqxYAReDgq	GDFEKCFAtp xx
Consensus	GRCK-DDFVE	GLSFARSG--	GNWEECFA-- --
Fcp10	GRCKRDDFVE	GLSFARSG..	GNWEECFA.. ..

Fig. 4d

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CP-1  
 Eco RI M G V F V V L L S I A T L F G S T 17  
 TATATGAATTCATGGGCGTGTTCGTCGTGCTACTGTCCATTGCCACCTTGTTTCGGTTCCA  
 1 -----+-----+-----+-----+-----+-----+ 60  
 ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGAACAAGCCAAGGT  
  
 S G T A L G P R G N S H S C D T V D G G 37  
 CATCCGGTACCGCCTTGGGTCCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG  
 61 -----+-----+-----+-----+-----+-----+ 120  
 GTAGGCCATGGCGGAACCCAGGAGCACCATTAAAGAGTGAGAACACTGTGACAACCTGCCAC

CP-2  
 CP-3.10  
 Y Q C F P E I S H L W G Q Y S P F F S L 57  
 GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATTCTTCTCTT  
 121 -----+-----+-----+-----+-----+-----+ 180  
 CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCAGTTATGAGAGGTAAGAAGAGAA  
  
 A D E S A I S P D V P K G C R V T F V Q 77  
 TGGCTGACGAATCTGCTATTTCTCCAGACGTTCCAAAGGGTGTAGAGTTACTTTTCGTTC  
 181 -----+-----+-----+-----+-----+-----+ 240  
 ACCGACTGCTTAGACGATAAAGAGGTCTGCAAGGTTTCCCGACATCTCAATGAAAGCAAG

CP-4.10  
 CP-5.10  
 V L S R H G A R Y P T S S K S K K Y S A 97  
 AAGTTTTGTCTAGACACGGTGCTAGATACCCAACTTCTTCTAAGTCTAAGAAGTACTCTG  
 241 -----+-----+-----+-----+-----+-----+ 300  
 TTCAAAACAGATCTGTGCCACGATCTATGGGTTGAAGAAGATTCAGATTCTTCATGAGAC  
  
 L I E A I Q K N A T A F K G K Y A F L K 117  
 CTTTGATTGAAGCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA  
 301 -----+-----+-----+-----+-----+-----+ 360  
 GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAAGT

CP-6  
 CP-7.10  
 T Y N Y T L G A D D L T P F G E Q Q M V 137  
 AGACTTACAACCTACACTTTGGGTGCTGACGACTTGACTCCATTCCGGTGAACAACAAATGG  
 361 -----+-----+-----+-----+-----+-----+ 420  
 TCTGAATGTTGATGTGAAACCCACGACTGCTGAAGTGAAGGTAAGCCACTTGTTGTTTACC  
  
 N S G I K F Y R R Y K A L A R K I V P F 157  
 TTAAGTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT  
 421 -----+-----+-----+-----+-----+-----+ 480  
 AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA

CP-8.10  
 CP-9.10  
 V R A S G S D R V I A S A E K F I E G F 177  
 TCGTTAGAGCTTCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT  
 481 -----+-----+-----+-----+-----+-----+ 540  
 AGCAATCTCGAAGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA  
  
 Q S A K L A D P G A N P H Q A S P V I N 197  
 TCCAATCTGCTAAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTA  
 541 -----+-----+-----+-----+-----+-----+ 600  
 AGGTTAGACGATTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAAT

Fig. 5a

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CP-10.10  
CP-11.10  
V I I P E G A G Y N N T L D H G L C T A 217  
ACGTTATTATTCCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTG  
601 -----+-----+-----+-----+-----+-----+ 660  
TGCAATAATAAGGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGAC

F E E S E L G D D V E A N F T A V F A P 237  
CTTTCGAAGAATCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTCGCTC  
661 -----+-----+-----+-----+-----+-----+ 720  
GAAAGCTTCTTAGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAG

CP-12.10  
P I R A R L E A H L P G V N L T D E D V 257  
CACCTATTAGAGCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACG  
721 -----+-----+-----+-----+-----+-----+ 780  
GTGGATAATCTCGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGC

CP-13.10  
V N L M D M C P F D T V A R T S D A T Q 277  
TTGTTAACTTGATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTC  
781 -----+-----+-----+-----+-----+-----+ 840  
AACAAATTGAACTACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAG

L S P F C D L F T H D E W I Q Y D Y L Q 297  
AATTGTCTCCATTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGC  
841 -----+-----+-----+-----+-----+-----+ 900  
TTAACAGAGGTAAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACG

CP-14.10  
CP-15.10  
S L G K Y Y G Y G A G N P L G P A Q G V 317  
AATCTTTGGGTAAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTG  
901 -----+-----+-----+-----+-----+-----+ 960  
TTAGAAACCCATTTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTGAGTTCCAC

G F V N E L I A R L T H S P V Q D H T S 337  
TTGGTTTCGTTAACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTT  
961 -----+-----+-----+-----+-----+-----+ 1020  
AACCAAAGCAATTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAA

CP-16.10  
CP-17.10  
T N H T L D S N P A T F P L N A T L Y A 357  
CTACTAACCACACTTTGGACTCTAACCAGCTACTTTCCCATTTGAACGCTACTTTGTACG  
1021 -----+-----+-----+-----+-----+-----+ 1080  
GATGATTGGTGTGAAACCTGAGATTGGGTGATGAAAGGGTAACCTTGCGATGAAACATGC

D F S H D N T M V S I F F A L G L Y N G 377  
CTGACTTCTCTCACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACG  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GACTGAAGAGAGTGCTGTTGTGATACCAAAGATAAAGAAGCGAAACCCAAACATGTTGC

CP-18.10  
CP-19.10  
T K P L S T T S V E S I E E T D G Y A A 397  
GTACTAAGCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACGCTG  
1141 -----+-----+-----+-----+-----+-----+ 1200  
CATGATTGCGTAACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGCGAC

Fig. 5b



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	1	50
<i>P. involutus</i> (phyA1)	----- -FPipeseqR nWSPYSPYFP LAEyKA....	pPaGCQInqV
<i>P. involutus</i> (phyA2)	----- -FsipeseqR nWSPYSPYFP LAEyKA....	pPaGCeInqV
<i>T. pubescens</i>	----- -LDvtRDVqQ sWSmYSPYFP aAtyvA....	pPaSCQInqV
<i>A. pediades</i>	----- -pffpPQIqD sWAaYTPYYP VqAyTP....	pPKDCKITqV
<i>P. lycii</i>	----- -LPipAQnTs nWGPYdPFFP VEpyAA....	pPEGctVTqV
<i>A. terreus</i> 9a1	KhsdCNSVDh GYQCfPELSH kWGLYAPYFS LqDESPFP1D	VPEDCHITfV
<i>A. terreus</i> cbs	NhsdCtSVDr GYQCfPELSH kWGLYAPYFS LqDESPFP1D	VPDDCHITfV
<i>A. niger</i> var. <i>awamori</i>	NqsTCDTVdQ GYQCfSEtSH LWGQYAPFFS LANESAISPD	VPaGCRVTfA
<i>A. niger</i> T213	NqsSCDTVdQ GYQCfSEtSH LWGQYAPFFS LANESvISPD	VPaGCRVTfA
<i>A. niger</i> NRRL3135	NqsSCDTVdQ GYQCfSEtSH LWGQYAPFFS LANESvISPE	VPaGCRVTfA
<i>A. fumigatus</i> ATCC13073	GSkSCDTVd1 GYQCSPatSH LWGQYSPFFS LEDElSVSSK	LPKDCRITLV
<i>A. fumigatus</i> ATCC32722	GSkSCDTVd1 GYQCSPatSH LWGQYSPFFS LEDElSVSSK	LPKDCRITLV
<i>A. fumigatus</i> ATCC58128	GSkSCDTVd1 GYQCSPatSH LWGQYSPFFS LEDElSVSSK	LPKDCRITLV
<i>A. fumigatus</i> ATCC26906	GSkSCDTVd1 GYQCSPatSH LWGQYSPFFS LEDElSVSSK	LPKDCRITLV
<i>A. fumigatus</i> ATCC32239	GSkACDTVEl GYQCSPGtSH LWGQYSPFFS LEDElSVSSD	LPKDCRVTFV
<i>E. nidulans</i>	QNHSCNTaDg GYQCfPNVSH VWGQYSPYFS IEQESAISeD	VPhGCeVTFV
<i>T. thermophilus</i>	DSHSCNTVEg GYQCrPEISH sWGQYSPFFS LADQSEISPD	VPQNCKITfV
<i>T. lanuginosus</i>	----- -nvDIAR hWGQYSPFFS LAEvSEISPA	VPKGCRVeFV
<i>M. thermophila</i>	ESRPCDTpDl GFQCgTAISH FWGQYSPYFS VPSElDaS..	IPDDCeVTFa
Consensus Seq. 11	NSHSCDTVd- GYQC-PEISH LWGQYSPFFS LADESAISPD	VPKGCRVTfV
	51	100
<i>P. involutus</i> (phyA1)	NIIqRHGARF PTSGaTtRik AgLtKLQgvq nftDAKFnFI	KSFKYdLGns
<i>P. involutus</i> (phyA2)	NIIqRHGARF PTSGaAtRik AgLsKLQsvq nftDPKFDFI	KSftYdLGts
<i>T. pubescens</i>	HIIqRHGARF PTSGaAKRiq TaVAKLKaaS nytDPlLAFV	tnYtYSLGqD
<i>A. pediades</i>	NIIqRHGARF PTSGaGtRiq AaVKKLQsak TytDPRLDfL	tnYtYTLGhD
<i>P. lycii</i>	NLIqRHGARW PTSGarsRqv AaVAKIQmar PftDPKYEFL	NdFvYkFGvA
<i>A. terreus</i> 9a1	QVLARHGARS PThSKTKaYA AtIAaIQKSA TaFpGKYAFL	QSYNYSldSE
<i>A. terreus</i> cbs	QVLARHGARS PTdSKTKaYA AtIAaIQKNA TaLpGKYAFL	KSYNYSMGSE
<i>A. niger</i> var. <i>awamori</i>	QVLSRHGARY PTeSKGKKYS ALIEeIQQNv TtFDGKYAFL	KTYNYSLGAD
<i>A. niger</i> T213	QVLSRHGARY PTeSKGKKYS ALIEeIQQNv TtFDGKYAFL	KTYNYSLGAD
<i>A. niger</i> NRRL3135	QVLSRHGARY PTdSKGKKYS ALIEeIQQNA TtFDGKYAFL	KTYNYSLGAD
<i>A. fumigatus</i> ATCC13073	QVLSRHGARY PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> ATCC32722	QVLSRHGARY PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> ATCC58128	QVLSRHGARY PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> ATCC26906	QVLSRHGARY PTSSKSKKYk kLVtaIQaNA TdFKGKFAFL	KTYNYTLGAD
<i>A. fumigatus</i> ATCC32239	QVLSRHGARY PTASKSKKYk kLVtaIQKNA TeFKGKFAFL	ETYNyTLGAD
<i>E. nidulans</i>	QVLSRHGARY PTeSKSKaYS GLIEaIQKNA TsFwGQYAFL	ESYNYTLGAD
<i>T. thermophilus</i>	QLLSRHGARY PTSSKTElyS qLIeRIQKtA TaYKGyYAFL	KdYrYqLGAN
<i>T. lanuginosus</i>	QVLSRHGARY PTAhKSEvYA ELLQRIQDtA TeFKGDFAFL	RdYaYhLGAD
<i>M. thermophila</i>	QVLSRHGARA PtlkRAasYv DLIDRIHhGA isYgPgYEFL	RTYDYTLGAD
Consensus Seq. 11	QVLSRHGARY PTSSKSKKYS ALIERIQKNA T-FKGKYAFL	KTYNYTLGAD

Fig. 6a

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	101		150
<i>P. involutus</i> (phyA1)	DLvPFGAaQs fDAGqEaFaR YskLvSKNnL	PFIRAdGSDR	VVDSAtNWtA
<i>P. involutus</i> (phyA2)	DLvPFGAaQs fDAGLEvFaR YskLvSsDnL	PFIRSdGSDR	VVDTAtNWtA
<i>T. pubescens</i>	sLveLGAtQs sEAGqEaFtR YsSLvSaDeL	PFVRASGSDR	VVATANNWtA
<i>A. pediades</i>	DLvPFGAlQs sQAGeEtFQR YsfLvSKEnL	PFVRASSSNR	VVDSAtNWtE
<i>P. lycii</i>	DLlPFGANQs hQTGTdMYtR YsTLfEgGdV	PFVRAAGdQR	VVDSStNWtA
<i>A. terreus</i> 9a1	ELTPFGrNQL rDlGaQFYeR YNAL.TRHIn	PFVRATDAsR	VhESAeKFVE
<i>A. terreus</i> cbs	NLTPFGrNQL qDlGaQFYRR YDTL.TRHIn	PFVRAADSsR	VhESAeKFVE
<i>A. niger</i> var. <i>awamori</i>	DLTPFGEQEL VNSGIKFYQR YESL.TRNII	PFIRSSGSsR	VIASGEKFIE
<i>A. niger</i> T213	DLTPFGEQEL VNSGIKFYQR YESL.TRNII	PFIRSSGSsR	VIASGEKFIE
<i>A. niger</i> NRRL3135	DLTPFGEQEL VNSGIKFYQR YESL.TRNIV	PFIRSSGSsR	VIASGKKFIE
<i>A. fumigatus</i> ATCC13073	DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
<i>A. fumigatus</i> ATCC32722	DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
<i>A. fumigatus</i> ATCC58128	DLTPFGEQQL VNSGIKFYQR YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
<i>A. fumigatus</i> ATCC26906	DLTAFGEQQL VNSGIKFYQR YKAL.ARSVV	PFIRASGSDR	VIASGEKFIE
<i>A. fumigatus</i> ATCC32239	DLTPFGEQQM VNSGIKFYQK YKAL.AgSVV	PFIRSSGSsR	VIASGEKFIE
<i>E. nidulans</i>	DLTiFGENQM VDSGaKFYRR YKnL.ARKnt	PFIRASGSDR	VVASAEKFIn
<i>T. thermophilus</i>	DLTPFGENQM IQlGIKFYnH YKSL.ARNnV	PFVRCsGSDR	VIASGrIFIE
<i>T. lanuginosus</i>	NLTRFGEEQM MESGrQFYHR YREq.AREIV	PFVRAAGSAR	VIASAEfFnR
<i>M. thermophila</i>	ELTRtGQQQM VNSGIKFYRR YRAL.ARKsI	PFVRTAGqDR	VVhSAENftQ
Consensus Seq. 11	DLTPFGENQM VNSGIKFYRR YKAL-ARNIV	PFVRASGSDR	VIASAEKFIE
	151		200
<i>P. involutus</i> (phyA1)	GFaSA..... ..shNtvqPk LNLILPQ..T	gNDTLEDNMC	PAaGD.....
<i>P. involutus</i> (phyA2)	GFaSA..... ..srNaiqPk LDLILPQ..T	gNDTLEDNMC	PAaGE.....
<i>T. pubescens</i>	GFaLA..... ..ssNsITPV LSVIISE..A	gNDTLDDNMC	PAaGD.....
<i>A. pediades</i>	GFsAA..... ..shHvlnPI LfVILSE..S	LNDTLDDAMC	PnaGs.....
<i>P. lycii</i>	GFgdA..... ..sgEtv1Pt LQVVLQE..E	gNcTLcNNMC	PnevD.....
<i>A. terreus</i> 9a1	GFQTARqDDh hAnpHQPSPr VDVAIPEGSA	YNNTLEHSLC	TAFES...ST
<i>A. terreus</i> cbs	GFQNaRqGDP hAnpHQPSPr VDVVIPEGTA	YNNTLEHSIC	TAFEA...ST
<i>A. niger</i> var. <i>awamori</i>	GFQSTKLkDP rAqpgQSSPk IDVVISeASS	sNNTLDpGtC	TvFED...Se
<i>A. niger</i> T213	GFQSTKLkDP rAqpgQSSPk IDVVISeASS	sNNTLDpGtC	TvFED...Se
<i>A. niger</i> NRRL3135	GFQSTKLkDP rAqpgQSSPk IDVVISeASS	sNNTLDpGtC	TvFED...Se
<i>A. fumigatus</i> ATCC13073	GFQqAKLADP gAt.NRAAPa ISVIIPESeT	FNNTLDHGVC	TkFEA...Sq
<i>A. fumigatus</i> ATCC32722	GFQqAKLADP gAt.NRAAPa ISVIIPESeT	FNNTLDHGVC	TkFEA...Sq
<i>A. fumigatus</i> ATCC58128	GFQqAKLADP gAt.NRAAPa ISVIIPESeT	FNNTLDHGVC	TkFEA...Sq
<i>A. fumigatus</i> ATCC26906	GFQqAKLADP gAt.NRAAPa ISVIIPESeT	FNNTLDHGVC	TkFEA...Sq
<i>A. fumigatus</i> ATCC32239	GFQqANVADP gAt.NRAAPV ISVIIPESeT	YNNTLDHSVC	TnFEA...Se
<i>E. nidulans</i>	GFRkaQLhDh g.s.gQATPV VNVIIEIdG	FNNTLDHStC	vSFEN...de
<i>T. thermophilus</i>	GFQSAKVlDP hSdKHDApPt INVIIeEGPS	YNNTLDtGSc	PvFED...SS
<i>T. lanuginosus</i>	GFQdAKdrDP rSnkDQAePV INVIISeETG	sNNTLDgltC	PAaEE...AP
<i>M. thermophila</i>	GFHSALLADR gStvRPTlPy dmVVIPETAG	aNNTLHNDLC	TAFEEgpyST
Consensus Seq. 11	GFQSAKLADP -A--HQASPV INVIIPEGSG	YNNTLDHGVC	TAFED---ST

Fig. 6b

19/56

	201		250
<i>P. involutus</i> (phyA1)	.SDpqvnaWl AVafPSItAR LNAAApsvNL TDtDafNLVs LCAFlTVSK.		
<i>P. involutus</i> (phyA2)	.SDpqvDaWl AsafPSvtAQ LNAAApgANL TDADafNLVs LCPFmTVSK.		
<i>T. pubescens</i>	.SDpqvnQWl AqFAPPMtAR LNagaPGaNL TDtDtyNLLt LCPFETVAt.		
<i>A. pediades</i>	.SDpqtGiWT SIYGTPIanR LNqqaPGaNI TAADVsnLIp LCAFETivK.		
<i>P. lycii</i>	.GDESt.tWl GVfAPnItAR LNAAApsaNL SDsDaLtLMD MCPFDTLss.		
<i>A. terreus</i> 9a1	VGDDAvANFT AVFAPAIaqR LEAdLPGVQL StDDVVNLMA MCPFETVSlT		
<i>A. terreus</i> cbs	VGDAADNFT AVFAPAIakR LEAdLPGVQL SADDVVNLMA MCPFETVSlT		
<i>A. niger</i> var. <i>awamori</i>	LADtveANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIstS		
<i>A. niger</i> T213	LADtveANFT AtFAPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIstS		
<i>A. niger</i> NRRL3135	LADtveANFT AtFvPSIRqR LEndLSGVtL TDtEVtyLMD MCSFDTIstS		
<i>A. fumigatus</i> ATCC13073	LGDEvAANFT ALFAPdIRAR aEkhlPGVtL TDEDVVSLMD MCSFDTVART		
<i>A. fumigatus</i> ATCC32722	LGDEvAANFT ALFAPdIRAR aEkhlPGVtL TDEDVVSLMD MCSFDTVART		
<i>A. fumigatus</i> ATCC58128	LGDEvAANFT ALFAPdIRAR aEkhlPGVtL TDEDVVSLMD MCSFDTVART		
<i>A. fumigatus</i> ATCC26906	LGDEvAANFT ALFAPdIRAR aKkhLPGVtL TDEDVVSLMD MCSFDTVART		
<i>A. fumigatus</i> ATCC32239	LGDEvEANFT ALFAPAIRAR IEkhLPGVQL TDDDVVSLMD MCSFDTVART		
<i>E. nidulans</i>	rADEiEANFT AIMGPPIRkR LEndLPGIKL TNENViyLMD MCSFDTMART		
<i>T. thermophilus</i>	gGHDAQEKFA kqFAPAILEK IKDhLPGVDL AvsDVpyLMD LCPFETLARN		
<i>T. lanuginosus</i>	.DptqpAEFl qVFGPRVlKk ItkhMPGVNL TlEDVplFMD LCPFDTVGsd		
<i>M. thermophila</i>	IGDDAQDtYl StFAGPItAR VNanLPGaNL TDADtVaLMD LCPFETVAss		
Consensus Seq. 11	LGDDAEANFT AVFAPPiRAR LEA-LPGVNL TDEDVVNLMD MCPFDTVART		
	251		300
<i>P. involutus</i> (phyA1)	..... ekkSdF CtLFegIPGs FeaFAYggdL dKFYGTgyGQ		
<i>P. involutus</i> (phyA2)	..... eqkSdF CtLFegIPGs FeaFAYagdL dKFYGTgyGQ		
<i>T. pubescens</i>	..... errSeF CDIYeelqAE .daFAYnadL dKFYGTgyGQ		
<i>A. pediades</i>	..... etpSPF CNLF..TPEE FaQFEYFgdL dKFYGTgyGQ		
<i>P. lycii</i>	..... gnaSPF CDLF..TAAE YvsYEYYdL dKYYGTGPGN		
<i>A. terreus</i> 9a1	dD..Aht... ..LSPF CDLF..TAtE WtQYNYLlSL dKYYGYGGGN		
<i>A. terreus</i> cbs	dD..Aht... ..LSPF CDLF..TAAE WtQYNYLlSL dKYYGYGGGN		
<i>A. niger</i> var. <i>awamori</i>	Tv..DTK... ..LSPF CDLF..ThDE WiHYDYlQSL kKYYGHGAGN		
<i>A. niger</i> T213	Tv..DTK... ..LSPF CDLF..ThDE WiHYDYlRSL kKYYGHGAGN		
<i>A. niger</i> NRRL3135	Tv..DTK... ..LSPF CDLF..ThDE WiNYDYlQSL kKYYGHGAGN		
<i>A. fumigatus</i> ATCC13073	SD..ASQ... ..LSPF CQLF..ThNE WkKYNyLQSL gKYYGYGAGN		
<i>A. fumigatus</i> ATCC32722	SD..ASQ... ..LSPF CQLF..ThNE WkKYNyLQSL gKYYGYGAGN		
<i>A. fumigatus</i> ATCC58128	SD..ASQ... ..LSPF CQLF..ThNE WkKYNyLQSL gKYYGYGAGN		
<i>A. fumigatus</i> ATCC26906	SD..ASQ... ..LSPF CQLF..ThNE WkKYNyLQSL gKYYGYGAGN		
<i>A. fumigatus</i> ATCC32239	AD..ASE... ..LSPF CAIF..ThNE WkKYDYlQSL gKYYGYGAGN		
<i>E. nidulans</i>	AH..GTE... ..LSPF CAIF..TEKE WlQYDYlQSL sKYYGYGAGS		
<i>T. thermophilus</i>	ht..DT.... ..LSPF CALs..TqEE WqaYDYyQSL gKYYGnGGGN		
<i>T. lanuginosus</i>	PvlfPrQ... ..LSPF CHLF..TADD WmaYDYyTL dKYYSHGGGS		
<i>M. thermophila</i>	SsdpATadag ggngRpLSPF CrLF..SEsE WraYDYlQSV gKWYGYGPGN		
Consensus Seq. 11	SD--ATQ--- -----LSPF CDLF--TADE W-QYDYlQSL -KYYGYGAGN		

Fig. 6c

20/56

	301		350
<i>P. involutus</i> (phyA1)	eLGPvQGVGY vNELIARLTN	S.AVRDNTqT	NRTLDA SPvT FPLNkTFYAD
<i>P. involutus</i> (phyA2)	ALGPvQGVGY iNELLARLTN	S.AVNDNTqT	NRTLDAApDT FPLNkTMYAD
<i>T. pubescens</i>	PLGPvQGVGY iNELIARLTa	q.nVsDHTqT	NsTLDS SPET FPLNrTLYAD
<i>A. pediades</i>	PLGPvQGVGY iNELLARLTE	m.PVRDNTqT	NRTLDS SPIT FPLDrSIYAD
<i>P. lycii</i>	ALGPvQGVGY vNELLARLTg	q.AVRDETqT	NRTLDS DPAT FPLNrTFYAD
<i>A. terreus</i> 9a1	PLGPvQGVGW aNELMARLTR	A.PVHDHTCv	NNTLDA SPAT FPLNATLYAD
<i>A. terreus</i> cbs	PLGPvQGVGW aNELIARLTR	S.PVHDHTCv	NNTLDA NPAT FPLNATLYAD
<i>A. niger</i> var. <i>awamori</i>	PLGPTQGVGY aNELIARLTH	S.PVHDDTSS	NHTLDS NPAT FPLNSTLYAD
<i>A. niger</i> T213	PLGPTQGVGY aNELIARLTH	S.PVHDDTSS	NHTLDS NPAT FPLNSTLYAD
<i>A. niger</i> NRRL3135	PLGPTQGVGY aNELIARLTH	S.PVHDDTSS	NHTLDS SPAT FPLNSTLYAD
<i>A. fumigatus</i> ATCC13073	PLGPAQGIGF tNELIARLTR	S.PVQDHTST	NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> ATCC32722	PLGPAQGIGF tNELIARLTR	S.PVQDHTST	NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> ATCC58128	PLGPAQGIGF tNELIARLTR	S.PVQDHTST	NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> ATCC26906	PLGPAQGIGF tNELIARLTR	S.PVQDHTST	NsTLvSNPAT FPLNATMYvD
<i>A. fumigatus</i> ATCC32239	PLGPAQGIGF tNELIARLTN	S.PVQDHTST	NsTLDS DPAT FPLNATIYvD
<i>E. nidulans</i>	PLGPAQGIGF tNELIARLTQ	S.PVQDNTST	NHTLDS NPAT FPLDrkLYAD
<i>T. thermophilus</i>	PLGPAQGVGF vNELIARMTH	S.PVQDYTTv	NHTLDS NPAT FPLNATLYAD
<i>T. lanuginosus</i>	AFGPSRGVGF vNELIARMTg	NlPVKDHTTv	NHTLDdNPET FPLDAvLYAD
<i>M. thermophila</i>	PLGPTQGVGF vNELLARLA.	GvPVRDgTST	NRTL DGDP rT FPLGrPLYAD
Consensus Seq. 11	PLGPAQGVGF -NELIARLTH	S-PVQDHTST	NHTLDS NPAT FPLNATLYAD
	351		400
<i>P. involutus</i> (phyA1)	FSHDNlMVAV FsAMGLFrqP	aPLSTSvpNP	wrt.....Wr TSSlVPFSGR
<i>P. involutus</i> (phyA2)	FSHDNlMVAV FsAMGLFrqS	aPLSTSTpDP	nrt.....Wl TSSvVPFSAR
<i>T. pubescens</i>	FSHDNqMVAI FsAMGLFNqS	aPLdPTTpDP	art.....Fl vkkiVPFSAR
<i>A. pediades</i>	LSDHNqMIAI FsAMGLFNqS	sPLdPSfpNP	krt.....Wv TSRLtPFSAR
<i>P. lycii</i>	FSHDNTMVPI FaALGLFNAT	a.LdPlkpDe	nrl.....Wv DSklVPFSGH
<i>A. terreus</i> 9a1	FSHDSnLVSI FWALGLYNGT	aPLSqtSVES	Vs..QTDGYA AAWTVPFAAR
<i>A. terreus</i> cbs	FSHDSnLVSI FWALGLYNGT	KPLSqtTTVEd	It..rTDGYA AAWTVPFAAR
<i>A. niger</i> var. <i>awamori</i>	FSHDNGIISI LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
<i>A. niger</i> T213	FSHDNGIISI LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
<i>A. niger</i> NRRL3135	FSHDNGIISI LFALGLYNGT	KPLSTTTVEN	It..QTDGFS SAWTVPFASR
<i>A. fumigatus</i> ATCC13073	FSHDNSMVISI FFALGLYNGT	EPLSrTSVES	ak..ElDGYS ASWvVPFGAR
<i>A. fumigatus</i> ATCC32722	FSHDNSMVISI FFALGLYNGT	gPLSrTSVES	ak..ElDGYS ASWvVPFGAR
<i>A. fumigatus</i> ATCC58128	FSHDNSMVISI FFALGLYNGT	EPLSrTSVES	ak..ElDGYS ASWvVPFGAR
<i>A. fumigatus</i> ATCC26906	FSHDNSMVISI FFALGLYNGT	EPLSrTSVES	ak..ElDGYS ASWvVPFGAR
<i>A. fumigatus</i> ATCC32239	FSHDNGMIPi FFAMGLYNGT	EPLSqtSeES	tk..ESNGYS ASWAVPFGAR
<i>E. nidulans</i>	FSHDNSMISI FFAMGLYNGT	QPLSmdSVES	Iq..EmDGYA ASWTVPFGAR
<i>T. thermophilus</i>	FSHDNTMtSI FaALGLYNGT	akLSTTeIKS	Ie..ETDGYS AAWTVPFGGR
<i>T. lanuginosus</i>	FSHDNTMtGI FsAMGLYNGT	KPLSTSKIQP	ptgaAADGYA ASWTVPFAAR
<i>M. thermophila</i>	FSHDNdMMGV LgALGaYDgv	pPLdkTArrd	..peElGGYA ASWAVPFAAR
Consensus Seq. 11	FSHDNTMVSI FFALGLYNGT	KPLSTTSVES	I---ETDGYS ASWTVPFAAR

Fig. 6d

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	401		450
<i>P. involutus</i> (phyA1)	mvVERLsC.. fGt..... Tk	VRVLVQDQVq	PLEfCGgDRn
<i>P. involutus</i> (phyA2)	maVERLsC.. AGt..... Tk	VRVLVQDQVq	PLEfCGgDQd
<i>T. pubescens</i>	mvVERLDC.. GGa..... Qs	VRLLVNDaVq	PLafCGaDts
<i>A. pediades</i>	mvtErLLCQr DGtGsGGpsr imrNgnvQTF	VRILVNDaLq	PLkfCGgDmd
<i>P. lycii</i>	mtVEkLaC.. .....sgKea	VRVLVNDaVq	PLEfCGg.vd
<i>A. terreus</i> 9a1	AYVEMMQCrA .....EK...EPL	VRVLVNDRVm	PLHGCPtDKL
<i>A. terreus</i> cbs	AYIEMMQCrA .....EK...QPL	VRVLVNDRVm	PLHGCAVDNL
<i>A. niger</i> var. <i>awamori</i>	lYVEMMQCQA .....EQ...EPL	VRVLVNDRVV	PLHGCPIDaL
<i>A. niger</i> T213	lYVEMMQCQA .....EQ...EPL	VRVLVNDRVV	PLHGCPIDaL
<i>A. niger</i> NRRL3135	lYVEMMQCQA .....EQ...EPL	VRVLVNDRVV	PLHGCPVDaL
<i>A. fumigatus</i> ATCC13073	AYfEtMQCKS .....EK...EPL	VRaLINDRVV	PLHGCDVDKL
<i>A. fumigatus</i> ATCC32722	AYfEtMQCKS .....EK...EPL	VRaLINDRVV	PLHGCDVDKL
<i>A. fumigatus</i> ATCC58128	AYfEtMQCKS .....EK...ESL	VRaLINDRVV	PLHGCDVDKL
<i>A. fumigatus</i> ATCC26906	AYfEtMQCKS .....EK...EPL	VRaLINDRVV	PLHGCDVDKL
<i>A. fumigatus</i> ATCC32239	AYfEtMQCKS .....EK...EPL	VRaLINDRVV	PLHGCAVDKL
<i>E. nidulans</i>	AYfELMQCE. ....KK...EPL	VRVLVNDRVV	PLHGCAVDKF
<i>T. thermophilus</i>	AYIEMMQCDD .....sD...EPV	VRVLVNDRVV	PLHGCEVDsL
<i>T. lanuginosus</i>	AYVELLRcET ETsSeEEeEG ..ED...EPF	VRVLVNDRVV	PLHGCrVDRW
<i>M. thermophila</i>	iYVEkMRCsG GGgGgGGgEG ..rQekdEeM	VRVLVNDRVm	TLkGCGaDer
Consensus Seq. 11	AYVEMMQCEA GG-G-GG-EG --EK---EPL	VRVLVNDRVV	PLHGCGVDKL
	451		482
<i>P. involutus</i> (phyA1)	GlCtLAKFVE SqTFARSDga	GDFEKCFAts	a~
<i>P. involutus</i> (phyA2)	GlCaLDKFVE SqAYARSGga	GDFEKCLAtt	v~
<i>T. pubescens</i>	GvCtLDAFVE SqAYARNDge	GDFEKCFAt~	--
<i>A. pediades</i>	SlCtLEAFVE SqkYAREDgq	GDFEKCFD~	--
<i>P. lycii</i>	GvCELSAFVE SqTYARENgq	GDFAKCgfv	se
<i>A. terreus</i> 9a1	GRCKrDAFVA GLSFAQAG..	GNWADCF---	--
<i>A. terreus</i> cbs	GRCKrDDFVE GLSFARAG..	GNWAECF---	--
<i>A. niger</i> var. <i>awamori</i>	GRCtrDsFVr GLSFARSG..	GDWAECsA--	--
<i>A. niger</i> T213	GRCtrDsFVr GLSFARSG..	GDWAECFA--	--
<i>A. niger</i> NRRL3135	GRCtrDsFVr GLSFARSG..	GDWAECFA--	--
<i>A. fumigatus</i> ATCC13073	GRCKLNDFVK GLSWARSG..	GNWGECFS--	--
<i>A. fumigatus</i> ATCC32722	GRCKLNDFVK GLSWARSG..	GNWGECFS--	--
<i>A. fumigatus</i> ATCC58128	GRCKLNDFVK GLSWARSG..	GNWGECFS--	--
<i>A. fumigatus</i> ATCC26906	GRCKLNDFVK GLSWARSG..	GNWGECFS--	--
<i>A. fumigatus</i> ATCC32239	GRCKLKDFVK GLSWARSG..	GNSEQSFS--	--
<i>E. nidulans</i>	GRcLDDWVE GLNFARSG..	GNWktCFTl-	--
<i>T. thermophilus</i>	GRCKrDDFVr GLSFARqG..	GNWEGCYAas	e~
<i>T. lanuginosus</i>	GRCRrDEWIK GLTFARqG..	GHWDrCF---	--
<i>M. thermophila</i>	GmCtLErFIE SMAFARGN..	GKWDlCFA--	--
Consensus Seq. 11	GRCKLDDFVE GLSFARSG--	GNWAECFA--	--

Fig. 6e

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M G V F V V L L S I A T L F G S T S G T 20  
ATGGGCGTGTTTCGTCTGCTACTGTCCATTGCCACCTTGTTTCGGTTCACATCCGGTACC  
1 -----+-----+-----+-----+-----+-----+ 60  
TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAAACAAGCCAAGGTGTAGGCCATGG  
  
A L G P R G N S H S C D T V D G G Y Q C 40  
GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT  
61 -----+-----+-----+-----+-----+-----+ 120  
CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA  
  
F P E I S H L W G T Y S P Y F S L A D E 60  
TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA  
121 -----+-----+-----+-----+-----+-----+ 180  
AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT  
  
S A I S P D V P D D C R V T F V Q V L S 80  
TCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTCAAGTTTGTCT  
187 -----+-----+-----+-----+-----+-----+ 240  
AGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAGTTCAAAACAGA  
  
R H G A R Y P T S S A S K A Y S A L I E 100  
AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA  
241 -----+-----+-----+-----+-----+-----+ 300  
TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAAACTAATT  
  
A I Q K N A T A F K G K Y A F L K T Y N 120  
GCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC  
301 -----+-----+-----+-----+-----+-----+ 360  
CGATAAGTTTCTTGCATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG  
  
Y T L G A D D L T P F G E N Q M V N S G 140  
TACACTTTGGGTGCTGACGACTTGACTCCATTCGGTGAAAACCAAATGGTTAACTCTGGT  
361 -----+-----+-----+-----+-----+-----+ 420  
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA  
  
I K F Y R R Y K A L A R K I V P F I R A 160  
ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT  
421 -----+-----+-----+-----+-----+-----+ 480  
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA  
  
S G S D R V I A S A E K F I E G F Q S A 180  
TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCAATTGAAGGTTTCCAATCTGCT  
481 -----+-----+-----+-----+-----+-----+ 540  
AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAGGTTAGACGA  
  
K L A D P G S Q P H Q A S P V I N V I I 200  
AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT  
541 -----+-----+-----+-----+-----+-----+ 600  
TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTTCAAGAGGTCAATAATTGCACTAGTAA  
  
P E G S G Y N N T L D H G T C T A F E D 220  
CCAGAAGGATCCGGTTACAACAACACTTTGGACCACGGTACTTGTACTGCTTTCGAAGAC  
601 -----+-----+-----+-----+-----+-----+ 660  
GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTGCCATGAACATGACGAAAGCTTCTG

Fig. 7a

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S E L G D D V E A N F T A L F A P A I R 240  
TCTGAATTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTTCGCTCCAGCTATTAGA  
661 -----+-----+-----+-----+-----+-----+ 720  
AGACTTAATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTCGATAATCT

A R L E A D L P G V T L T D E D V V Y L 260  
GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG  
721 -----+-----+-----+-----+-----+-----+ 780  
CGATCTAACCTTCGACTGAACGGTCCACAATGAACTGACTGCTTCTGCAACAAATGAAC

M D M C P F D T V A R T S D A T E L S P 280  
ATGGACATGTGTCCATTCGACACTGTCGCTAGAACTTCTGACGCTACTGAATTGTCTCCA  
781 -----+-----+-----+-----+-----+-----+ 840  
TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT

F C A L F T H D E W I Q Y D Y L Q S L G 300  
TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT  
841 -----+-----+-----+-----+-----+-----+ 900  
AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA

K Y Y G Y G A G N P L G P A Q G V G F A 320  
AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGCT  
901 -----+-----+-----+-----+-----+-----+ 960  
TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCGA

N E L I A R L T H S P V Q D H T S T N H 340  
AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC  
961 -----+-----+-----+-----+-----+-----+ 1020  
TTGCTTAACCTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

T L D S N P A T F P L N A T L Y A D F S 360  
ACTTTGGACTCTAACCCAGCTACTTTCCCATTTGAACGCTACTTTGTACGCTGACTTCTCT  
1021 -----+-----+-----+-----+-----+-----+ 1080  
TGAAACCTGAGATTGGGTGCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

H D N T M I S I F F A L G L Y N G T K P 380  
CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACCAAGCCA  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGGTTTCGGT

L S T T S V E S I E E T D G Y S A S W T 400  
TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTACTCTGCTTCTTGGA  
1141 -----+-----+-----+-----+-----+-----+ 1200  
AACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

V P F A A R A Y V E M M Q C Q A E K E P 420  
GTTCCATTGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA  
1201 -----+-----+-----+-----+-----+-----+ 1260  
CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTTCGACTTTTCCTTGGT

L V R V L V N D R V V P L H G C A V D K 440  
TTGGTTAGAGTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTGTGCTGTTGACAAG  
1261 -----+-----+-----+-----+-----+-----+ 1320  
AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACCTGTTTC

Fig. 7b

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      L  G  R  C  K  R  D  D  F  V  E  G  L  S  F  A  R  S  G  G      460
      TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTTCGCTAGATCTGGTGGT
1321  -----+-----+-----+-----+-----+-----+-----+ 1380
      AACCCTATCTACATTCTCTCTGCTGAAGCAACTTCCAACAGAAAGCGATCTAGACCACCA

      N  W  A  E  C  F  A  *      467
      AACTGGGCTGAATGTTTCGCTTAA
1381  -----+-----+----- 1410
      TTGACCCGACTTACAAAGCGAATT

```

**Fig. 7c**

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M G V F V V L L S I A T L F G S T S G T 20  
ATGGGCGTGTTCGTTCGTGCTACTGTCCATTGCCACCTTGTTTCGGTTCACATCCGGTACC  
1 -----+-----+-----+-----+-----+-----+ 60  
TACCCGCACAAGCAGCACGATGACAGGTAACGGTGAACAAGCCAAGGTGTAGGCCATGG  
  
A L G P R G N S H S C D T V D G G Y Q C 40  
GCCTTGGGTCCTCGTGGTAACTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT  
61 -----+-----+-----+-----+-----+-----+ 120  
CGGAACCCAGGAGCACCATTGAGAGTGAGAACACTGTGACAACCTGCCACCAATGGTTACA  
  
F P E I S H L W G T Y S P F F S L A D E 60  
TTCCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTTGGCTGACGAA  
121 -----+-----+-----+-----+-----+-----+ 180  
AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT  
  
S A I S P D V P K G C R V T F V Q V L S 80  
TCTGCTATTTCTCCAGACGTTCCAAAGGGTGTAGAGTACTTTTCGTTCAAGTTTGTCT  
181 -----+-----+-----+-----+-----+-----+ 240  
AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA  
  
R H G A R Y P T S S A S K A Y S A L I E 100  
AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCGTACTCTGCTTTGATTGAA  
241 -----+-----+-----+-----+-----+-----+ 300  
TCTGTGCCACGATCTATGGGTGAAGAAGACGCAGATTCCGCATGAGACGAACTAACTT  
  
A I Q K N A T A F K G K Y A F L K T Y N 120  
GCTATTCAAAGAAGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC  
301 -----+-----+-----+-----+-----+-----+ 360  
CGATAAGTTTCTTGCATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG  
  
Y T L G A D D L T P F G E Q Q M V N S G 140  
TACACTTTGGGTGCTGACGACTTGACTCCATTTCGGTGAACAACAAATGGTTAACTCTGGT  
361 -----+-----+-----+-----+-----+-----+ 420  
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACCAATTGAGACCA  
  
I K F Y R R Y K A L A R K I V P F I R A 160  
ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT  
421 -----+-----+-----+-----+-----+-----+ 480  
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTCTAACAAGGTAAGTAATCTCGA  
  
S G S D R V I A S A E K F I E G F Q S A 180  
TCTGGTTCGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT  
481 -----+-----+-----+-----+-----+-----+ 540  
AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAGGTTAGACGA  
  
K L A D P G A N P H Q A S P V I N V I I 200  
AAGTTGGCTGACCCAGGTGCTAACCACACCAAGCTTCTCCAGTTATTAACGTTATTATT  
541 -----+-----+-----+-----+-----+-----+ 600  
TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAATTGCAATAATAA  
  
P E G A G Y N N T L D H G L C T A F E E 220  
CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTCGAAGAA  
601 -----+-----+-----+-----+-----+-----+ 660  
GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT

Fig. 8a

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S E L G D D V E A N F T A V F A P P I R 240  
TCTGAATTGGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTTCGCTCCACCAATTAGA  
661 -----+-----+-----+-----+-----+-----+ 720  
AGACTTAACCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT

A R L E A H L P G V N L T D E D V V N L 260  
GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACGTTGTAACTTG  
721 -----+-----+-----+-----+-----+-----+ 780  
CGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGCAACAATTGAAC

M D M C P F D T V A R T S D A T Q L S P 280  
ATGGACATGTGTCCATTTCGACACTGTTGCTAGAAGTTCTGACGCTACTCAATTGTCTCCA  
781 -----+-----+-----+-----+-----+-----+ 840  
TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT

F C D L F T H D E W I Q Y D Y L Q S L G 300  
TTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT  
841 -----+-----+-----+-----+-----+-----+ 900  
AAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA

K Y Y G Y G A G N P L G P A Q G V G F V 320  
AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT  
901 -----+-----+-----+-----+-----+-----+ 960  
TTCATGATGCCAATGCCACGACCATTTGGGTAACCCAGGTTCGAGTTCCACAACCAAAGCAA

N E L I A R L T H S P V Q D H T S T N H 340  
AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC  
961 -----+-----+-----+-----+-----+-----+ 1020  
TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG

T L D S N P A T F P L N A T L Y A D F S 360  
ACTTTGGACTCTAACCCAGCTACTTTCCCATTTGAACGCTACTTTGTACGCTGACTTCTCT  
1021 -----+-----+-----+-----+-----+-----+ 1080  
TGAAACCTGAGATTGGGTTCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA

H D N T M V S I F F A L G L Y N G T K P 380  
CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTCGGT

L S T T S V E S I E E T D G Y S A S W T 400  
TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGACT  
1141 -----+-----+-----+-----+-----+-----+ 1200  
AACAGATGATGAAGACAACTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA

V P F A A R A Y V E M M Q C E A E K E P 420  
GTTCCATTGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA  
1201 -----+-----+-----+-----+-----+-----+ 1260  
CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT

L V R V L V N D R V V P L H G C G V D K 440  
TTGGTTAGAGTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGGTGTGACAAG  
1261 -----+-----+-----+-----+-----+-----+ 1320  
AACCAATCTCAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACCACAACCTGTTC

Fig. 8b

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```

      L G R C K R D D F V E G L S F A R S G G      460
      TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT
1321 -----+-----+-----+-----+-----+-----+ 1380
      AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA

      N W E E C F A *      467
      AACTGGGAAGAATGTTTCGCTTAA
1381 -----+-----+----- 1404
      TTGACCCTTCTTACAAAGCGAATT
```

Fig. 8c

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M G V F V V L L S I A T L F G S T S G T 20  
ATGGGGGTTTTCGTCGTTCTATTATCTATCGCGACTCTGTTTCGGCAGCACATCGGGCACT  
1 -----+-----+-----+-----+-----+-----+ 60  
TACCCCAAAAGCAGCAAGATAATAGATAGCGCTGAGACAAGCCGTCGTGTAGCCCGTGA  
  
A L G P R G N H S K S C D T V D L G Y Q 40  
GCGCTGGGCCCCCGTGGAAATCACTCCAAGTCCTGCGATACGGTAGACCTAGGGTACCAG  
61 -----+-----+-----+-----+-----+-----+ 120  
CGCGACCCGGGGGCACCTTTAGTGAGGTTCAAGACGCTATGCCATCTGGATCCCATGGTC  
  
C S P A T S H L W G T Y S P Y F S L E D 60  
TGCTCCCCTGCGACTTCTCATCTATGGGGCACGTACTCGCCATaCTTTTCGCTCGAGGAC  
121 -----+-----+-----+-----+-----+-----+ 180  
ACGAGGGGACGCTGAAGAGTAGATAACCCGtgCATGAGCGGTAtGAAAAGCGAGCTCCTG  
  
E L S V S S K L P K D C R I T L V Q V L 80  
GAGCTGTCCGTGTGCGAGTAAGCTTCCCAAGGATTGCCGGATCACCTTGGTACAGGTGCTA  
181 -----+-----+-----+-----+-----+-----+ 240  
CTCGACAGGCACAGCTCATTCGAAGGGTTCCTAACGGCCTAGTGGAACCATGTCCACGAT  
  
S R H G A R Y P T S S K S K K Y K K L I 100  
TCGCGCCATGGAGCGCGGTACCCAACCAGCTCCAAGAGCAAAAAGTATAAGAAGCTTaTt  
241 -----+-----+-----+-----+-----+-----+ 300  
AGCGCGGTACCTCGCGCCATGGGTTGGTCGAGGTTCTCGTTTTTCATATTCTTCGAAtAa  
  
T A I Q A N A T D F K G K Y A F L K T Y 120  
ACGGCGATCCAGGCCAATGCCACCGACTTCAAGGGCAAGTAcGCCTTTTTGAAGACGTAC  
301 -----+-----+-----+-----+-----+-----+ 360  
TGCCGCTAGGTCCGGTTACGGTGGCTGAAGTTCCCGTTCatgCGGAAAACTTCTGCATG  
  
N Y T L G A D D L T P F G E Q Q L V N S 140  
AACTATACTCTGGGTGCGGATGACCTCACTCCCTTTGGGGAGCAGCAGCTGGTGAACCTCG  
361 -----+-----+-----+-----+-----+-----+ 420  
TTGATATGAGACCCACGCTACTGGAGTGAGGGAAACCCCTCGTCGTCGACCACTTGAGC  
  
G I K F Y Q R Y K A L A R S V V P F I R 160  
GGCATCAAGTTCTACCAGAGGTACAAGGCTCTGGCGCGCAGTGTGGTGCCGTTTATTTCGC  
421 -----+-----+-----+-----+-----+-----+ 480  
CCGTAGTTCAAGATGGTCTCCATGTTCCGAGACCGCGCGTCACACCACGGCAAATAAGCG  
  
A S G S D R V I A S G E K F I E G F Q Q 180  
GCCTCAGGCTCGGACCGGGTTATTGCTTCGGGAGAGAAGTTTCATCGAGGGGTTCCAGCAG  
481 -----+-----+-----+-----+-----+-----+ 540  
CGGAGTCCGAGCCTGGCCCAATAACGAAGCCCTCTCTTCAAGTAGCTCCCCAAGGTCGTC  
  
A K L A D P G A T N R A A P A I S V I I 200  
GCGAAGCTGGCTGATCCTGGCGCGACGAACCGCGCCGCTCCGGCGATTAGTGTGATTATT  
541 -----+-----+-----+-----+-----+-----+ 600  
CGCTTCGACCGACTAGGACCGCGCTGCTTGGCGCGGCGAGGCCGCTAATCACACTAATAA  
  
P E S E T F N N T L D H G V C T K F E A 220  
CCGGAGAGCGAGACGTTCAACAATACGCTGGACCACGGTGTGTGCACGAAGTTTGAGGCG  
601 -----+-----+-----+-----+-----+-----+ 660  
GGCCTCTCGCTCTGCAAGTTGTTATGCGACCTGGTGCCACACACGTGCTTCAAACCTCCGC

Fig. 9a

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S Q L G D E V A A N F T A L F A P D I R 240  
AGTCAGCTGGGAGATGAGGTTGCGGCCAATTTCACTGCGCTCTTTGCACCCGACATCCGA  
661 -----+-----+-----+-----+-----+-----+ 720  
TCAGTCGACCCTCTACTCCAACGCCGGTTAAAGTGACGCGAGAAACGTGGGCTGTAGGCT

A R L E K H L P G V T L T D E D V V S L 260  
GCTCGCctCGAGAAGCATCTTCCTGGCGTGACGCTGACAGACGAGGACGTTGTCAGTCTA  
721 -----+-----+-----+-----+-----+-----+ 780  
CGAGCGgaGCTCTTCGTAGAAGGACCGCACTGCGACTGTCTGCTCCTGCAACAGTCAGAT

M D M C P F D T V A R T S D A S Q L S P 280  
ATGGACATGTGTcCGTTTGATACGGTAGCGCGCACCAGCGACGCAAGTCAGCTGTCACCG  
781 -----+-----+-----+-----+-----+-----+ 840  
TACCTGTACACAgGCAAACCTATGCCATCGCGCGTGGTCGCTGCGTTCAGTCGACAGTGGC

F C Q L F T H N E W K K Y D Y L Q S L G 300  
TTCTGTCAACTCTTCACTCACAATGAGTGGAAGAAGTACgACTACCTTCAGTCCTTGGGC  
841 -----+-----+-----+-----+-----+-----+ 900  
AAGACAGTTGAGAAGTGAGTGTTACTCACCTTCTTCATGcTGATGGAAGTCAGGAACCCG

K Y Y G Y G A G N P L G P A Q G I G F T 320  
AAGTACTACGGCTACGGCGCAGGCAACCCTCTGGGACCGGCTCAGGGGATAGGGTTCACC  
901 -----+-----+-----+-----+-----+-----+ 960  
TTCATGATGCCGATGCCGCGTCCGTTGGGAGACCCTGGCCGAGTCCCCTATCCCAAGTGG

N E L I A R L T R S P V Q D H T S T N S 340  
AACGAGCTGATTGCCCCGGTTGACgCGTTCGCCAGTGACAGGACCACACCAGCACTAACTCG  
961 -----+-----+-----+-----+-----+-----+ 1020  
TTGCTCGACTAACGGGCCAACTGcGCAAGCGGTCACGTCCTGGTGTGGTCGTGATTGAGC

T L V S N P A T F P L N A T M Y V D F S 360  
ACTCTAGTCTCCAACCCGGCCACCTTCCCGTTGAACGCTACCATGTACGTCGACTTTTCA  
1021 -----+-----+-----+-----+-----+-----+ 1080  
TGAGATCAGAGGTTGGGCCGGTGGAAGGGCAACTTGCGATGGTACATGCAGCTGAAAAGT

H D N S M V S I F F A L G L Y N G T E P 380  
CACGACAACAGCATGGTTTCCATCTTCTTTGCATTGGGCCTGTACAACGGCACTGAACCC  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GTGCTGTTGTCTGATACCAAAGGTAGAAGAAACGTAACCCGGACATGTTGCCGTGACTTGGG

L S R T S V E S A K E L D G Y S A S W V 400  
TTGTCCCGGACCTCGGTGGAAAGCGCCAAGGAATTGGATGGGTATTCTGCATCCTGGGTG  
1141 -----+-----+-----+-----+-----+-----+ 1200  
AACAGGGCCTGGAGCCACCTTTTCGCGGTTCTTAACCTACCCATAAGACGTAGGACCCAC

V P F G A R A Y F E T M Q C K S E K E P 420  
GTGCCTTTTCGGCGCGCGAGCCTACTTCGAGACGATGCAATGCAAGTCGGAAAAGGAGCCT  
1201 -----+-----+-----+-----+-----+-----+ 1260  
CACGGAAAGCCGCGCGCTCGGATGAAGCTCTGCTACGTTACGTTTCAGCCTTTTCCTCGGA

L V R A L I N D R V V P L H G C D V D K 440  
CTTGTTTCGCGCTTTGATTAATGACCGGGTTGTGCCACTGCATGGCTGCGATGTGGACAAG  
1261 -----+-----+-----+-----+-----+-----+ 1320  
GAACAAGCGCGAAACTAATTACTGGCCCAACACGGTGACGTACCGACGCTACACCTGTTC

Fig. 9b

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```

      L  G  R  C  K  L  N  D  F  V  K  G  L  S  W  A  R  S  G  G      460
      CTGGGGCGATGCAAGCTGAATGACTTTGTCAAGGGATTGAGTTGGGCCAGATCTGGGGGC
1321 -----+-----+-----+-----+-----+-----+ 1380
      GACCCCGCTACGTTGACTTACTGAAACAGTTCCTAACTCAACCCGGTCTAGACCCCG

      N  W  G  E  C  F  S  *      467
      AACTGGGGAGAGTGCTTTAGTTGA
1381 -----+-----+----- 1404
      TTGACCCCTCTCACGAAATCAACT
```

Fig. 9c

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CP-1  
Eco RI M G V F V V L L S I A T L F G S T  
TATATGAATTCATGGGCGTGTTCGTGCTACTGTCCATTGCCACCTTGTTCCGGTTCCA  
1 -----+-----+-----+-----+-----+-----+ 60  
ATATACTTAAGTACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAAACAAGCCAAGGT  
  
S G T A L G P R G N S H S C D T V D G G  
CATCCGGTACCGCCTTGGGTCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTG  
61 -----+-----+-----+-----+-----+-----+ 120  
GTAGGCCATGGCGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCAC  
CP-2  
CP-3  
Y Q C F P E I S H L W G Q Y S P Y F S L  
GTTACCAATGTTTCCCAGAAATTTCTCACTTGTGGGGTCAATACTCTCCATACTTCTCTT  
121 -----+-----+-----+-----+-----+-----+ 180  
CAATGGTTACAAAGGGTCTTTAAAGAGTGAACACCCCAGTTATGAGAGGTATGAAGAGAA  
  
E D E S A I S P D V P D D C R V T F V Q  
TGGAAGACGAATCTGCTATTTCTCCAGACGTTCCAGACGACTGTAGAGTTACTTTCGTTT  
181 -----+-----+-----+-----+-----+-----+ 240  
ACCTTCTGCTTAGACGATAAAGAGGTCTGCAAGGTCTGCTGACATCTCAATGAAAGCAAG  
CP-4.7  
CP-5.7  
V L S R H G A R Y P T D S K G K K Y S A  
AAGTTTGTCTAGACACGGTGCTAGATACCCAAGTgacTCTAAGggtAAGaagTACTCTG  
241 -----+-----+-----+-----+-----+-----+ 300  
TTCAAAACAGATCTGTGCCACGATCTATGGGTTGActgAGATTCCcaTTcTtCATGAGAC  
  
L I E A I Q K N A T A F K G K Y A F L K  
CTTTGATTGAAGCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGA  
301 -----+-----+-----+-----+-----+-----+ 360  
GAAACTAACTTCGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACT  
CP-6  
CP-7  
T Y N Y T L G A D D L T P F G E N Q M V  
AGACTTACAACCTACACTTTGGGTGCTGACGACTTGACTCCATTCCGGTGAAAACCAAATGG  
361 -----+-----+-----+-----+-----+-----+ 420  
TCTGAATGTTGATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACC  
  
N S G I K F Y R R Y K A L A R K I V P F  
TTAACTCTGGTATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCAT  
421 -----+-----+-----+-----+-----+-----+ 480  
AATTGAGACCATAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTA  
CP-8.7  
CP-9  
I R A S G S S R V I A S A E K F I E G F  
TCATTAGAGCTTCTGGTTCTtctAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTT  
481 -----+-----+-----+-----+-----+-----+ 540  
AGTAATCTCGAAGACCAAGAagaTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAA  
  
Q S A K L A D P G S Q P H Q A S P V I D  
TCCAATCTGCTAAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTG  
541 -----+-----+-----+-----+-----+-----+ 600  
AGGTTAGACGATTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAAC

Fig. 10a

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CP-10.7

CP-11.7

V I I S E A S S Y N N T L D P G T C T A  
 ACGTTATTATTtctGAcgctTCTtctTACAACAACACTTTGGACccaGGTACTTGTACTG  
 601 -----+-----+-----+-----+-----+ 660  
 TGCAATAATAAagaCTgcgaAGGagaATGTTGTTGTGAAACCTGggtCCATGAACATGAC

F E D S E L A D T V E A N F T A L F A P  
 CTTTCGAAGACTCTGAATTGgctGACactGTTGAAGCTAACTTCACTGCTTTGTTTCGCTC  
 661 -----+-----+-----+-----+-----+ 720  
 GAAAGCTTCTGAGACTTAACcgaCTGtgaCAACTTCGATTGAAGTGACGAAACAAGCGAG

CP-12.7

A I R A R L E A D L P G V T L T D T E V  
 CAGCTATTAGAGCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACactgaaG  
 721 -----+-----+-----+-----+-----+ 780  
 GTCGATAATCTCGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGtgacttc

CP-13.7

T Y L M D M C S F E T V A R T S D A T E  
 TTactTACTTGATGGACATGTGTtctTTTCGAAACTGTTGCTAGAACTTCTGACGCTACTG  
 781 -----+-----+-----+-----+-----+ 840  
 AatgaATGAACTACCTGTACACAagaAAGCTTTGACAACGATCTTGAAGACTGCGATGAC

L S P F C A L F T H D E W R H Y D Y L Q  
 AATTGTCTCCATTCTGTGCTTTGTTCACTCACGACGAATGGAGAcactACGACTACTTGC  
 841 -----+-----+-----+-----+-----+ 900  
 TTAACAGAGGTAAGACACGAAACAAGTGAGTGCTGCTTACCTCTgtgATGCTGATGAACG

CP-14.7

CP-15.7

S L K K Y Y G H G A G N P L G P T Q G V  
 AATCTTTGaagAAGTACTACGGTcacGGTGCTGGTAACCCATTGGGTCCAactCAAGGTG  
 901 -----+-----+-----+-----+-----+ 960  
 TTAGAAACttcTTCATGATGCCAgtgCCACGACCATTGGGTAACCCAGGTtgaGTTCCAC

G F A N E L I A R L T R S P V Q D H T S  
 TTGGTTTCGCTAACGAATTGATTGCTAGATTGACTAGATCTCCAGTTCAAGACCACACTT  
 961 -----+-----+-----+-----+-----+ 1020  
 AACCAAAGCGATTGCTTAACCTAACGATCTAACTGATCTAGAGGTCAAGTTCTGGTGTGAA

CP-16

CP-17.7

T N H T L D S N P A T F P L N A T L Y A  
 CTACTAACCACACTTTGGACTCTAACCCAGCTACTTTCCCATTGAACGCTACTTTGTACG  
 1021 -----+-----+-----+-----+-----+ 1080  
 GATGATTGGTGTGAAACCTGAGATTGGGTCGATGAAAGGGTAACTTGCGATGAAACATGC

D F S H D N G I I S I F F A L G L Y N G  
 CTGACTTCTCTCACGACAACggtattATTCTATTTTCTTCGCTTTGGGTTTGTACAACG  
 1081 -----+-----+-----+-----+-----+ 1140  
 GACTGAAGAGAGTGCTGTTGccataaTAAAGATAAAAGAAGCGAAACCCAAACATGTTGC

CP-18.7

CP-19.7

T A P L S T T S V E S I E E T D G Y S S  
 GTACTGCTCCATTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTt  
 1141 -----+-----+-----+-----+-----+ 1200  
 CATGACGAGGTAACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGaa

Fig. 10b



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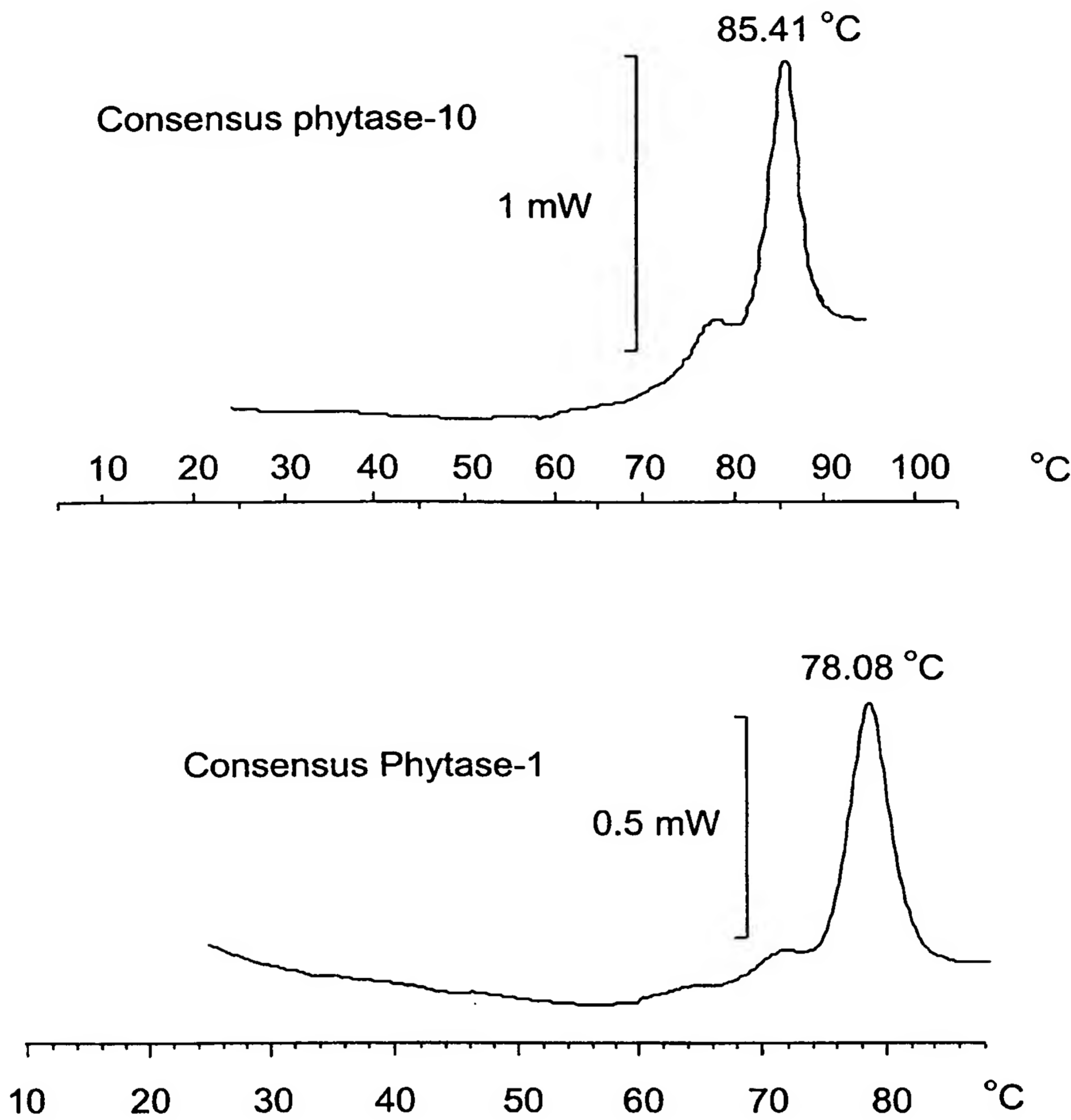


Fig. 11

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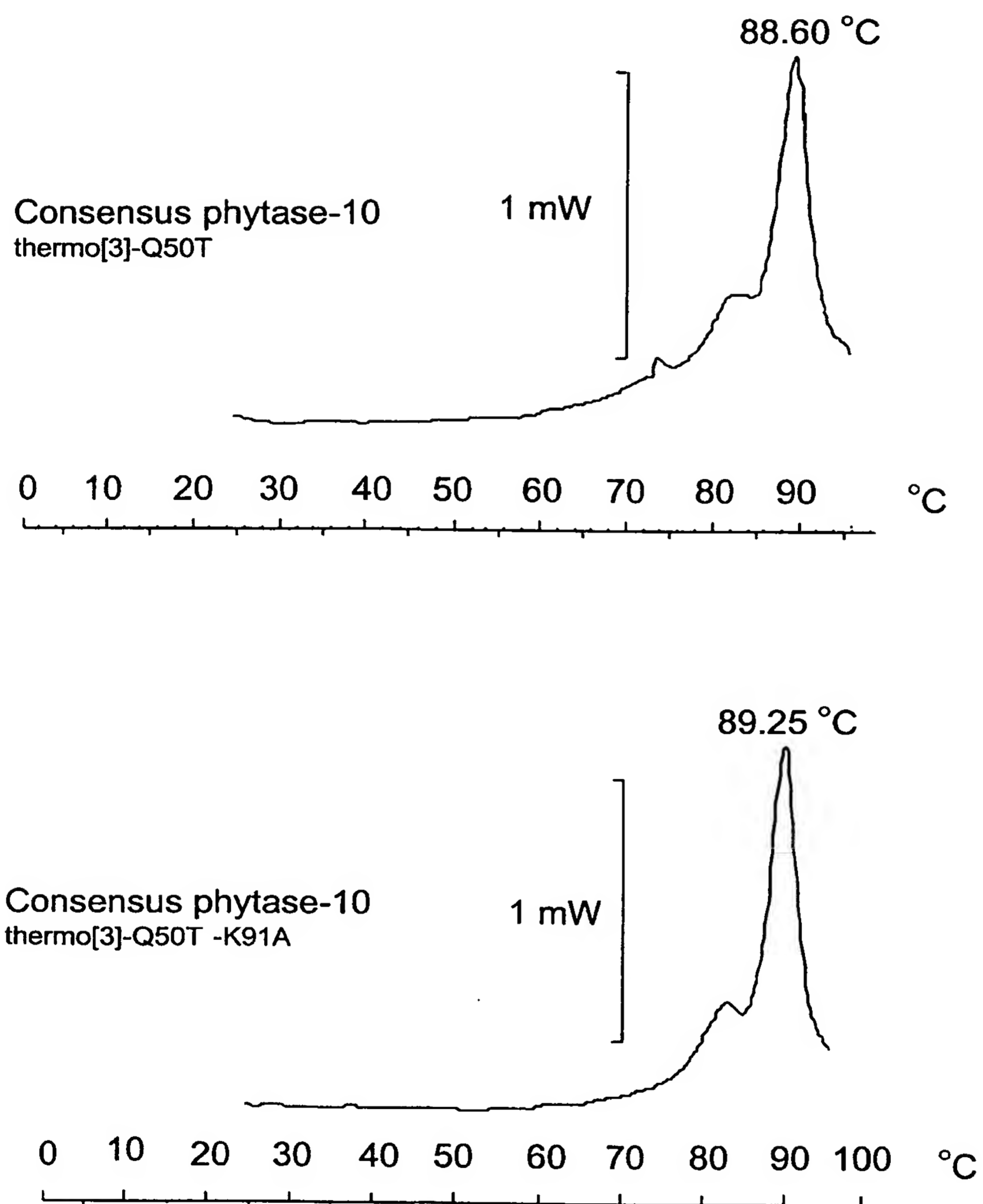


Fig. 12

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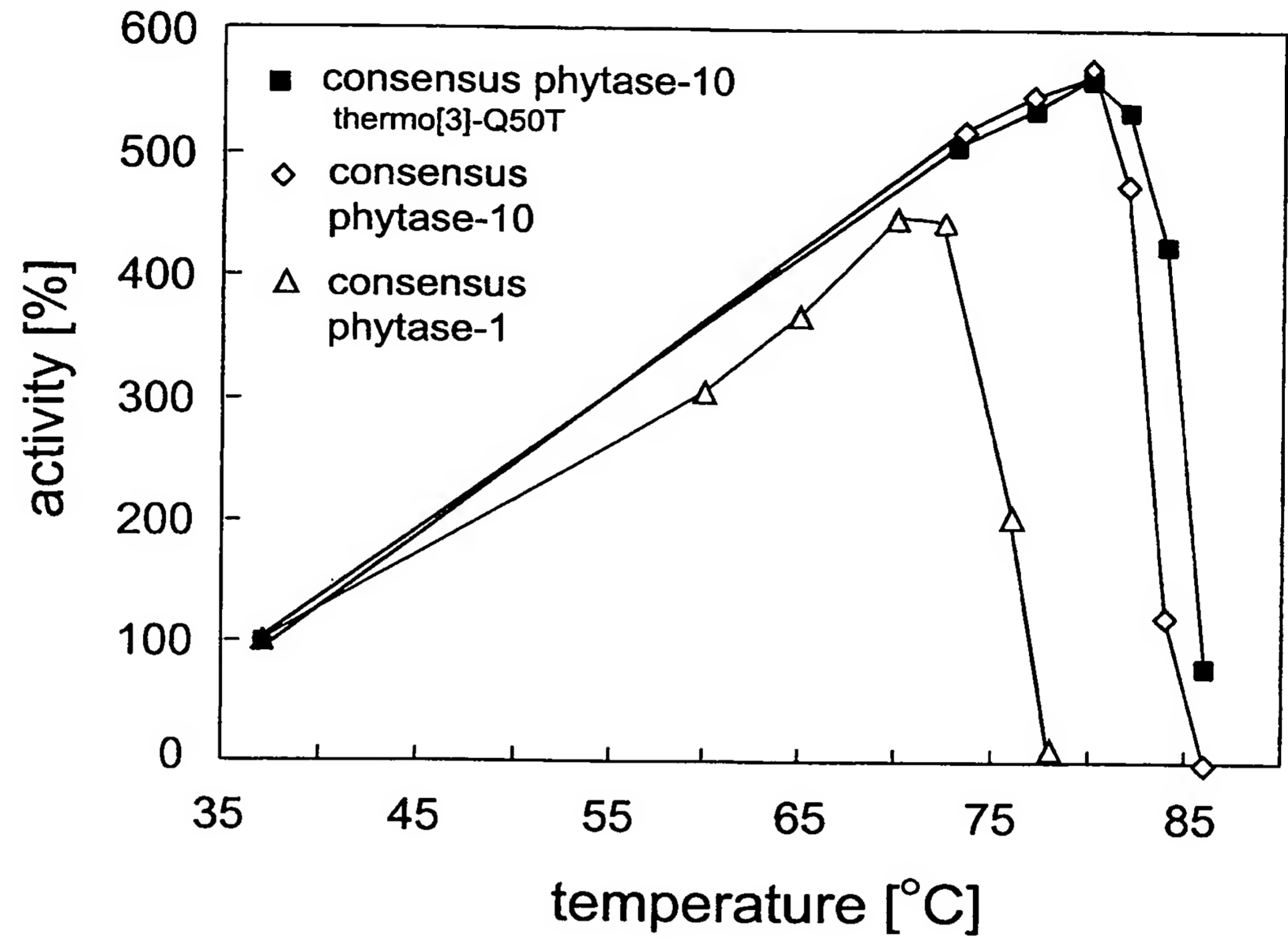


Fig. 13

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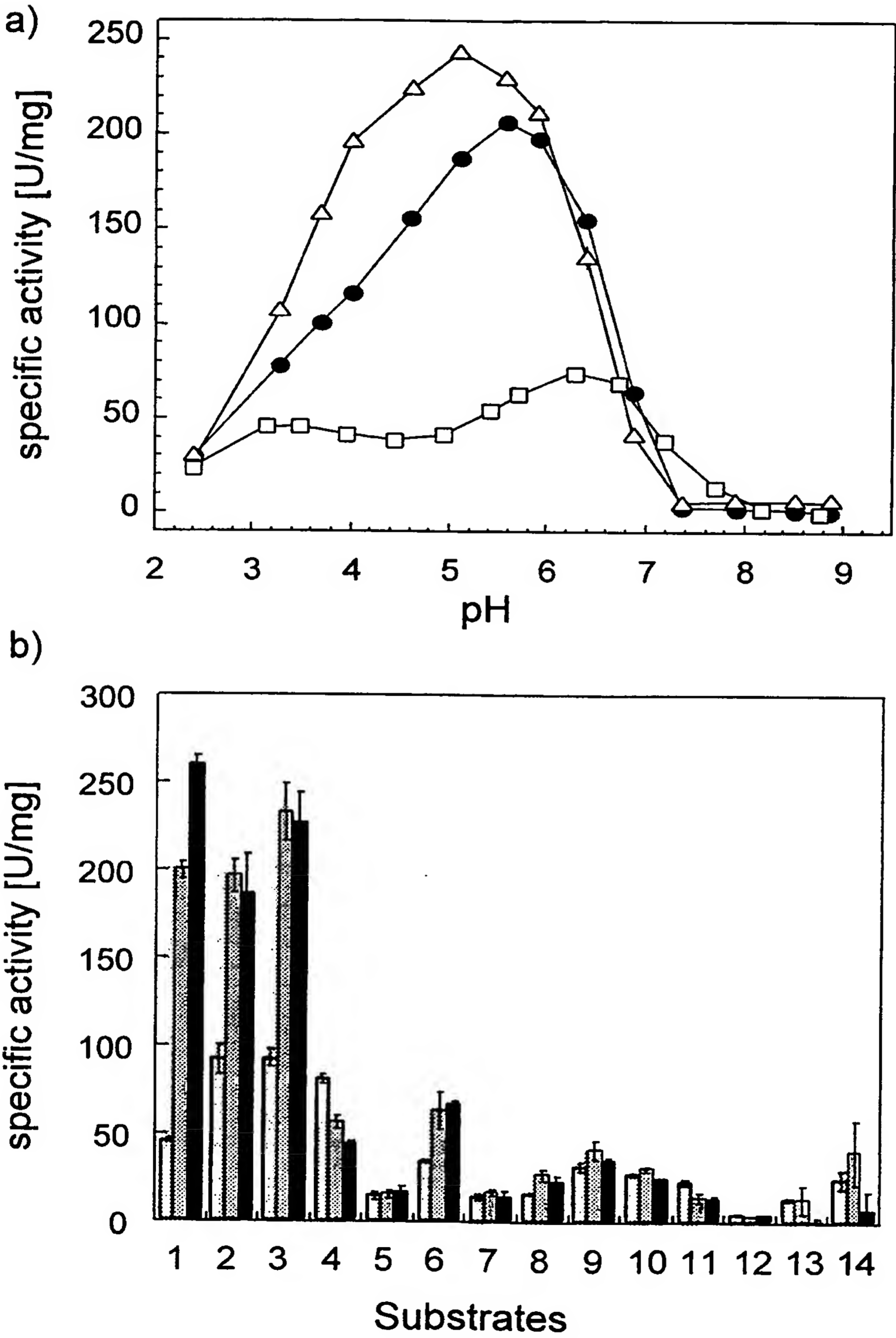


Fig. 14

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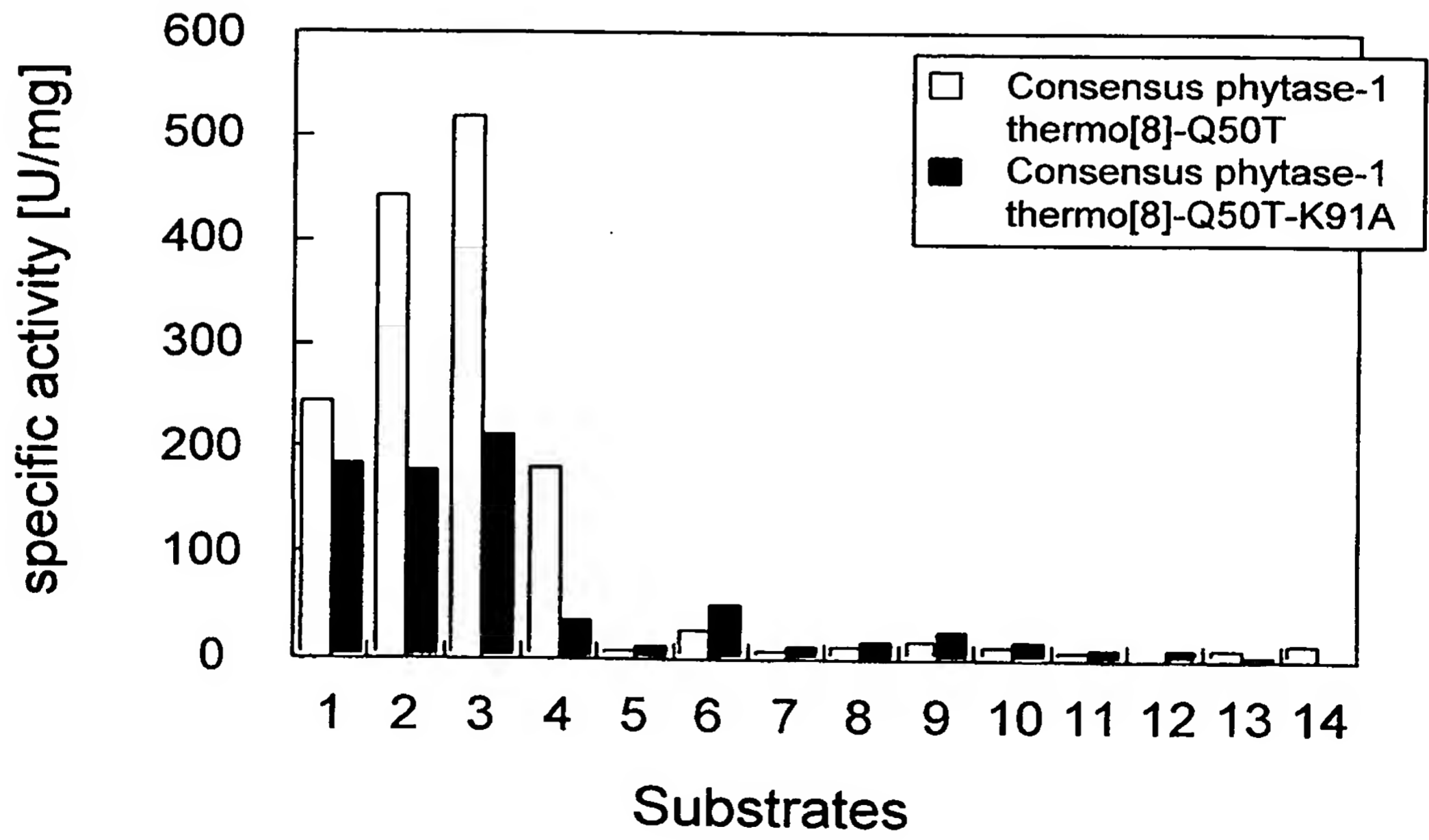
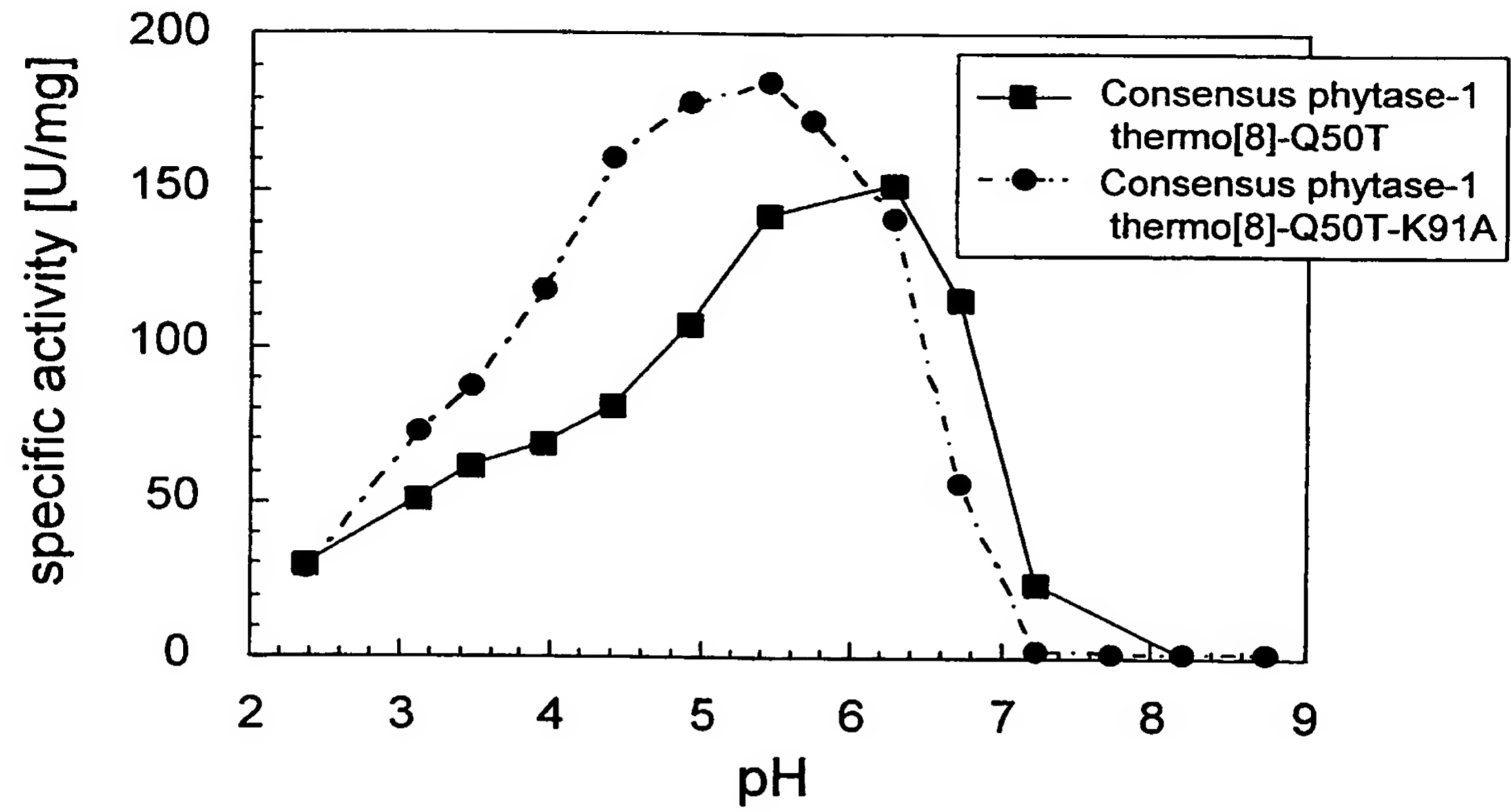


Fig. 15

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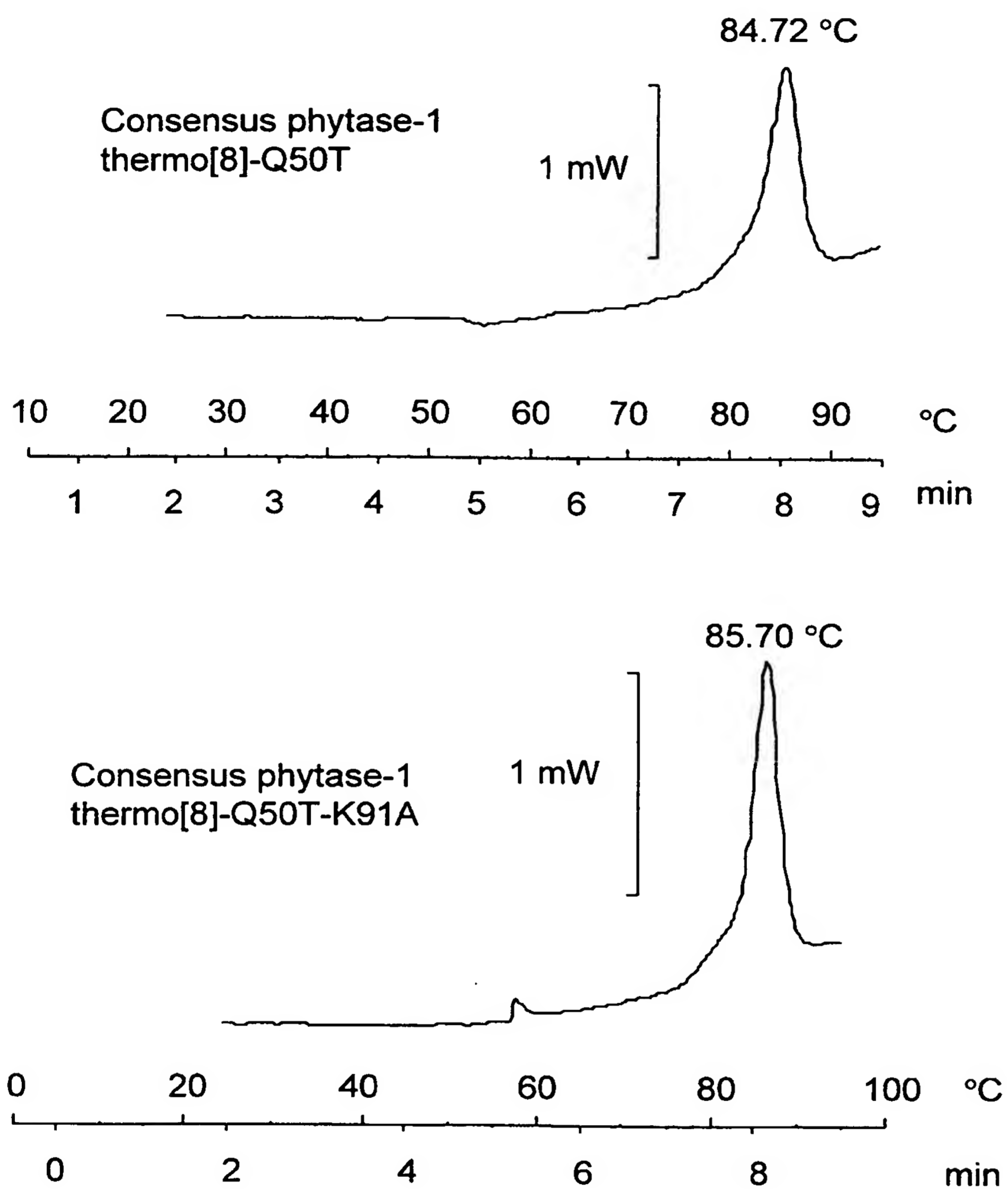


Fig. 16

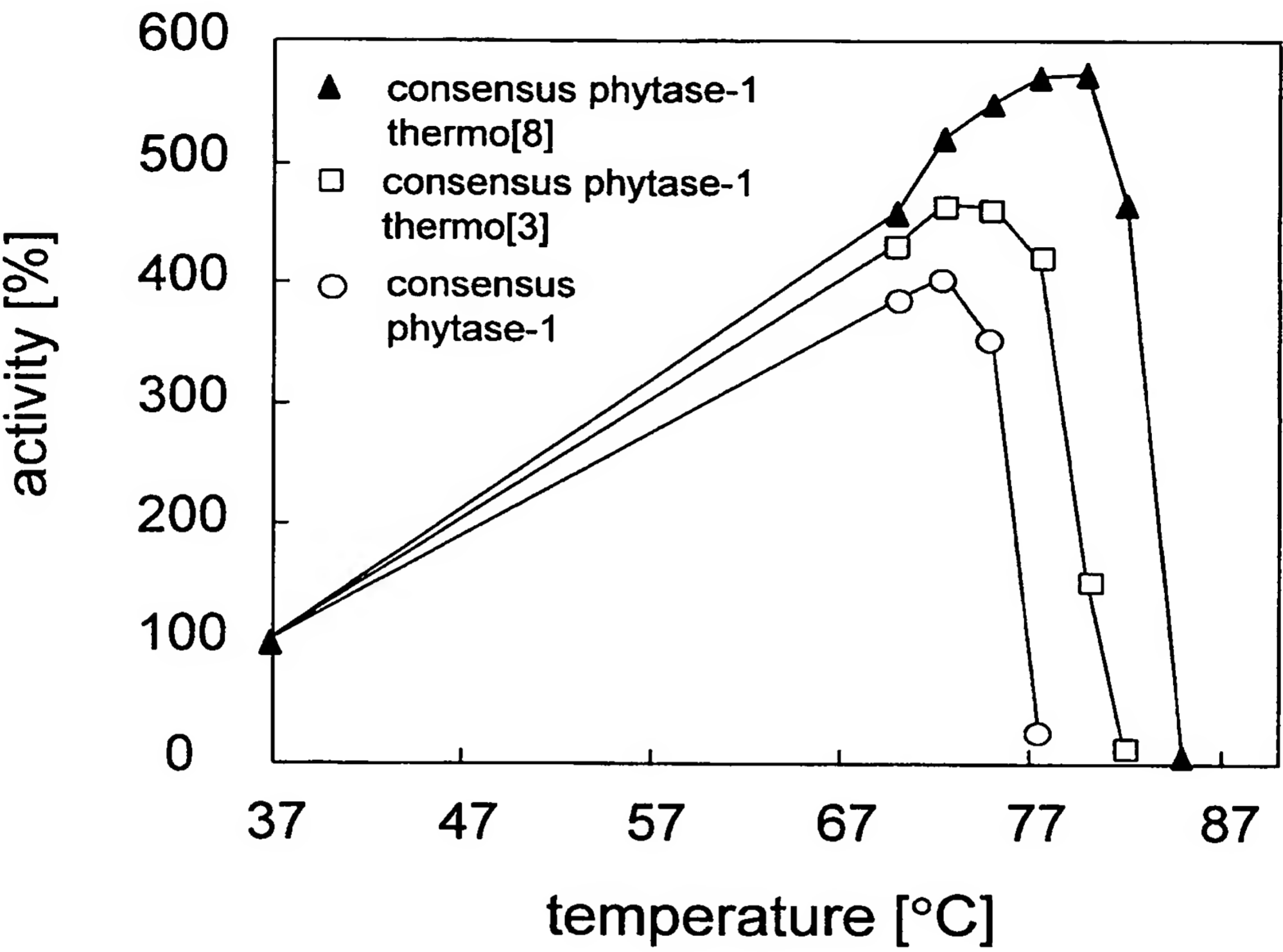


Fig. 17

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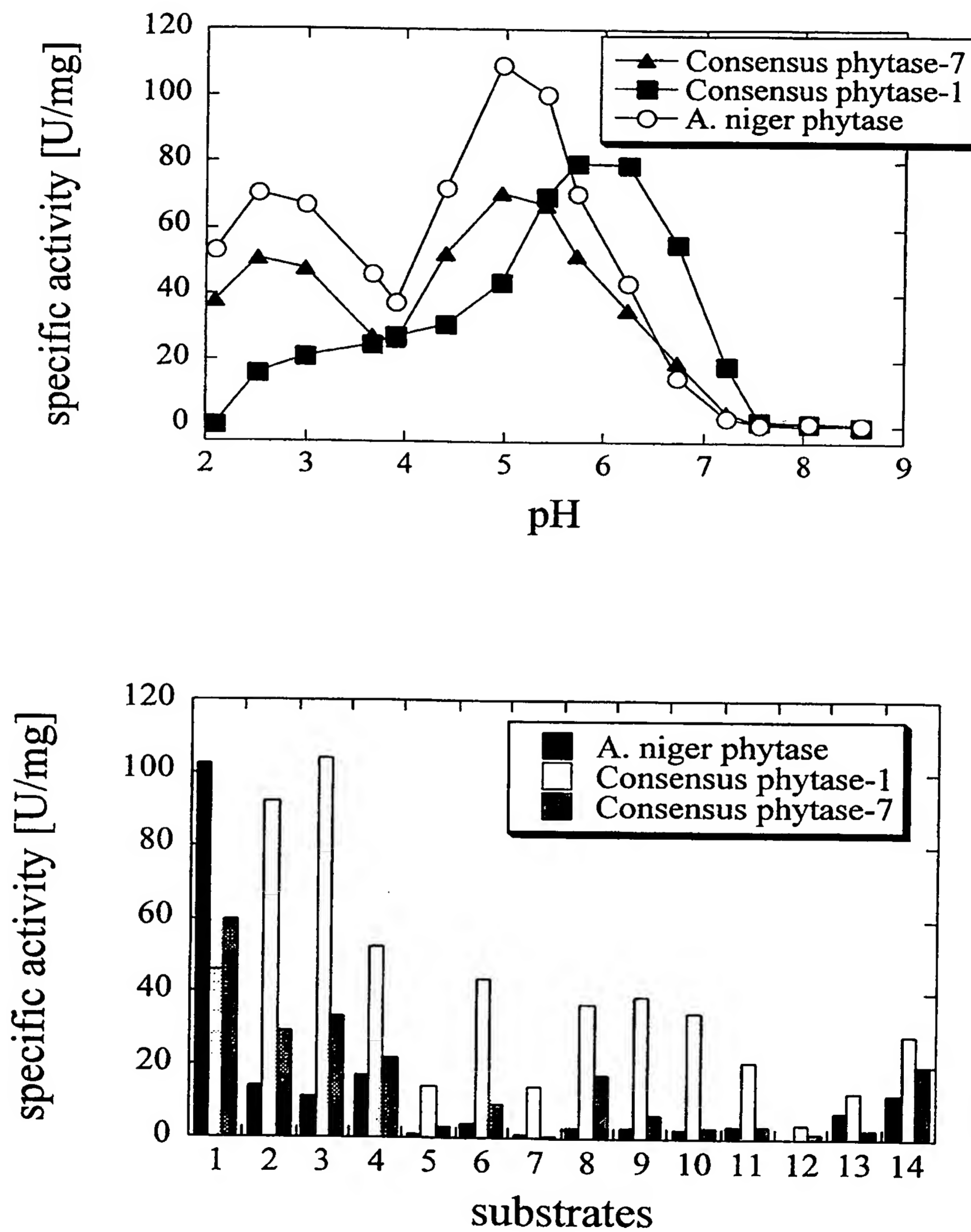


Fig. 18

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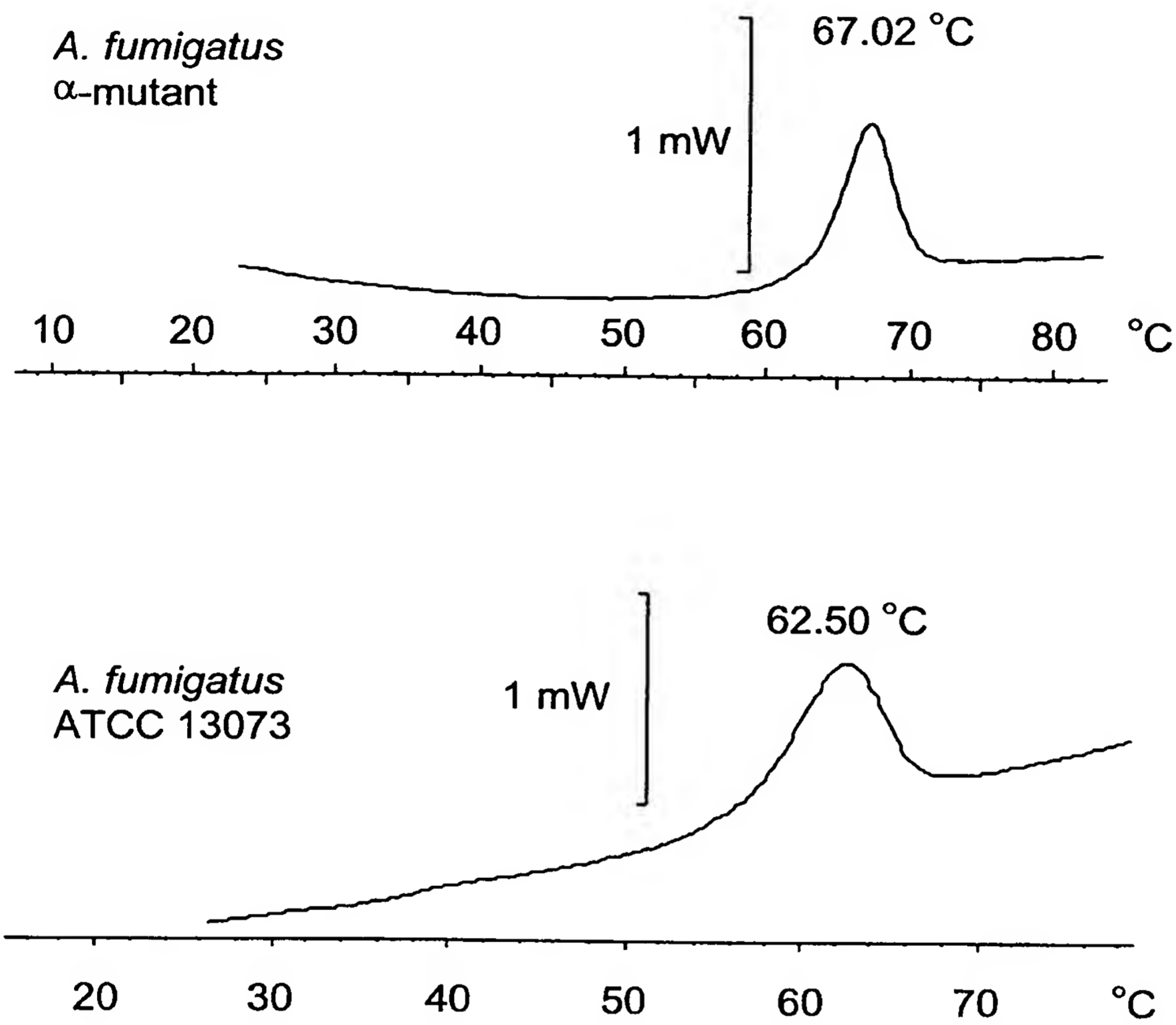


Fig. 19

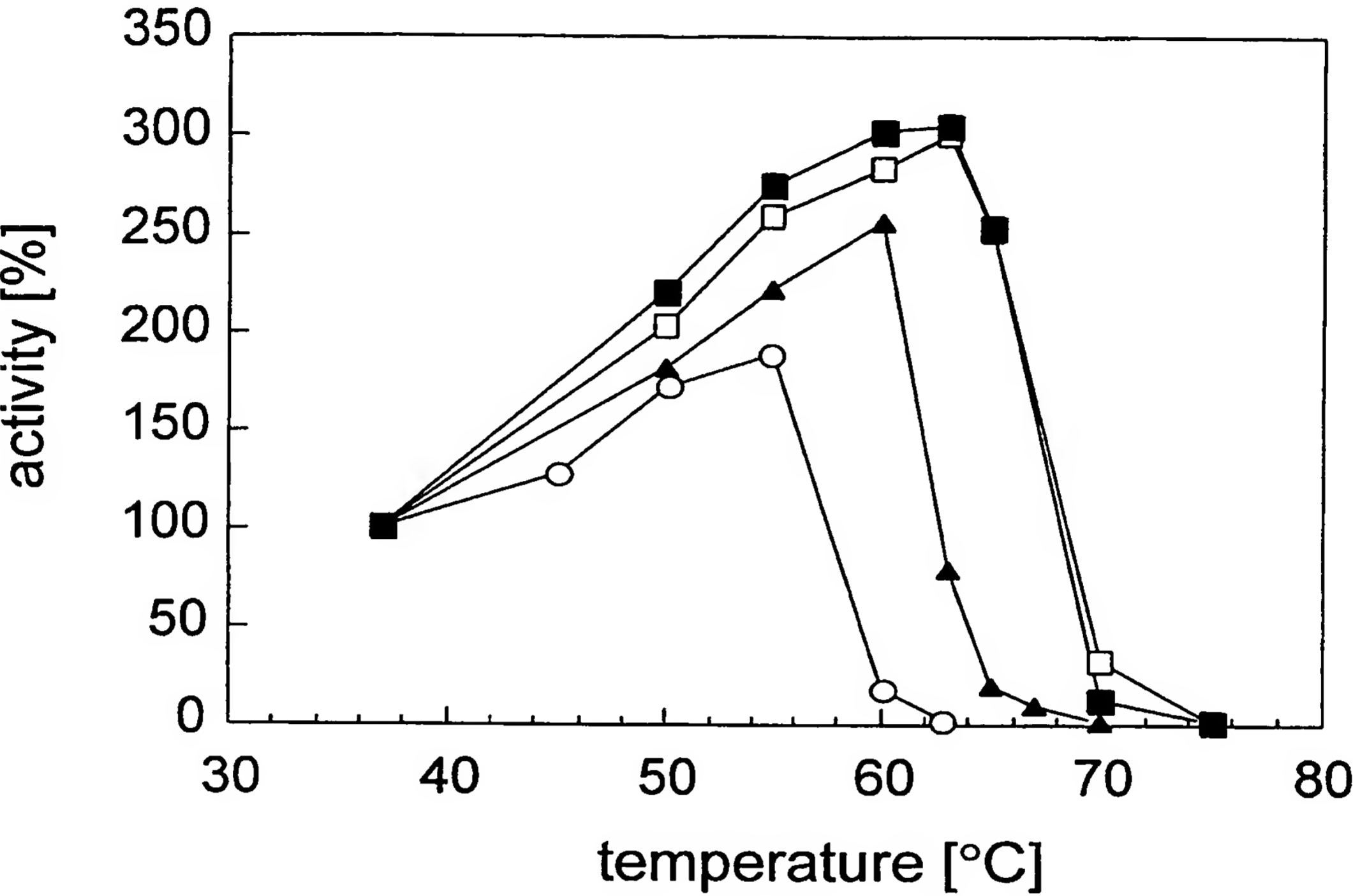


Fig. 20

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1     MGVFVLLSI  ATLFGSTSGT  ALGPRGNSHS  CDTVDGGYQC  FPEISSNWSP  
51    YSPYFSLADE  SAISPDVPKG  CRVTFVQVLQ  RHGARFPTSG  AATRISALIE  
101   AIQKNATAFK  GKYAFLKTYN  YTLGADDLVP  FGANQSSQAG  IKFYRRYKAL  
151   ARKIVPFIRA  SGSDRVIDSA  TNWIEGFQSA  KLADPGANPH  QASPVINVII  
201   PEGAGYNNTL  DHGLCTAFEE  SELGDDVEAN  FTAVFAPPIR  ARLEAHLPGV  
251   NLTDEDVVNL  MDMCPFDTVA  RTSDATEELSP  FCDLFTHDEW  IQYDYLGDLD  
301   KYYGTGAGNP  LGPAQGVGFV  NELIARLTHS  PVQDHTSTNH  TLDSNPATFP  
351   LNATLYADFS  HDNTMVAIFF  ALGLYNGTKP  LSTTSVESIE  ETDGYSASWL  
401   VPFSARMYVE  MMQCEAEKEP  LVRVLVNDRV  VPLHGCGVDK  LGRCKRDDFV  
451   EGLSFARSGG  NWEECFA

Fig. 21

45/56

ATGGGCGTGTTTCGTTCGTGCTACTGTCCATTGCCACCTTGTTTCGGTTCACATCCGGTACC  
1 -----+-----+-----+-----+-----+-----+-----+ 60  
TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAAACAAGCCAAGGTGTAGGCCATGG  
M G V F V V L L S I A T L F G S T S G T -  
GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT  
61 -----+-----+-----+-----+-----+-----+-----+ 120  
CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA  
A L G P R G N S H S C D T V D G G Y Q C -  
TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA  
121 -----+-----+-----+-----+-----+-----+-----+ 180  
AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT  
F P E I S H L W G T Y S P Y F S L A D E -  
TCTGCTATTTCTCCAGACGTCCCAAAGGACTGTAGAGTTACTTTCGTTCAAGTTTTGTCT  
181 -----+-----+-----+-----+-----+-----+-----+ 240  
AGACGATAAAGAGGTCTGCAGGGTTTCCTGACATCTCAATGAAAGCAAGTTCAAAACAGA  
S A I S P D V P K D C R V T F V Q V L S -  
AGACACGGTGCTAGATAACCAACTTCTTCTAAGTCTAAGGCTTACTCTGCTTTGATTGAA  
241 -----+-----+-----+-----+-----+-----+-----+ 300  
TCTGTGCCACGATCTATGGGTGGAAGAAGATTCAGATTCCGAATGAGACGAACTAACTT  
R H G A R Y P T S S K S K A Y S A L I E -  
GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC  
301 -----+-----+-----+-----+-----+-----+-----+ 360  
CGATAAGTTTTCTTGCATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG  
A I Q K N A T A F K G K Y A F L K T Y N -  
TACACTTTGGGTGCTGACGACTTGACTCCATTTCGGTGAAAACCAAATGGTTAACTCTGGT  
361 -----+-----+-----+-----+-----+-----+-----+ 420  
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA  
Y T L G A D D L T P F G E N Q M V N S G -  
ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT  
421 -----+-----+-----+-----+-----+-----+-----+ 480  
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA  
I K F Y R R Y K A L A R K I V P F I R A -  
TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT  
481 -----+-----+-----+-----+-----+-----+-----+ 540  
AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAAGGTTAGACGA  
S G S D R V I A S A E K F I E G F Q S A -

Fig. 22a

46/56

AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT  
541 -----+-----+-----+-----+-----+-----+ 600  
TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAATTGCACTAGTAA  
K L A D P G S Q P H Q A S P V I N V I I -  
CCAGAAGGATCCGGTTACAACAACACTTTGGACCATGGTCTTTGTACTGCTTTCGAAGAC  
601 -----+-----+-----+-----+-----+-----+ 660  
GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTACCAGAAACATGACGAAAGCTTCTG  
P E G S G Y N N T L D H G L C T A F E D -  
TCTACCCTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTTCGCTCCAGCTATTAGA  
661 -----+-----+-----+-----+-----+-----+ 720  
AGATGGGATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTCGATAATCT  
S T L G D D V E A N F T A L F A P A I R -  
GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG  
721 -----+-----+-----+-----+-----+-----+ 780  
CGATCTAACCTTCGACTGAACGGTCCACAATGAAACTGACTGCTTCTGCAACAAATGAAC  
A R L E A D L P G V T L T D E D V V Y L -  
ATGGACATGTGTCCATTCGACACTGTGCTAGAACTTCTGACGCTACTGAATTGTCTCCA  
781 -----+-----+-----+-----+-----+-----+ 840  
TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT  
M D M C P F D T V A R T S D A T E L S P -  
TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT  
841 -----+-----+-----+-----+-----+-----+ 900  
AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA  
F C A L F T H D E W I Q Y D Y L Q S L G -  
AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGCT  
901 -----+-----+-----+-----+-----+-----+ 960  
TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTGAGTTCCACAACCAAAGCGA  
K Y Y G Y G A G N P L G P A Q G V G F A -  
AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC  
961 -----+-----+-----+-----+-----+-----+ 1020  
TTGCTTAACCTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG  
N E L I A R L T H S P V Q D H T S T N H -  
ACTTTGGACTCTAACCCAGCTACTTTCCCATTTGAACGCTACTTTGTACGCTGACTTCTCT  
1021 -----+-----+-----+-----+-----+-----+ 1080  
TGAAACCTGAGATTGGGTGCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA  
T L D S N P A T F P L N A T L Y A D F S -

Fig. 22b

47/56

CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACCAAGCCA  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGGTTCGGT  
  
H D N T M I S I F F A L G L Y N G T K P -  
  
TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGGA  
1141 -----+-----+-----+-----+-----+-----+ 1200  
AACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA  
  
L S T T S V E S I E E T D G Y S A S W T -  
  
GTTCCATTTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA  
1201 -----+-----+-----+-----+-----+-----+ 1260  
CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTCGACTTTTCCTTGGT  
  
V P F A A R A Y V E M M Q C Q A E K E P -  
  
TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG  
1261 -----+-----+-----+-----+-----+-----+ 1320  
AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACCTGTTC  
  
L V R V L V N D R V V P L H G C A V D K -  
  
TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTTCGCTAGATCTGGTGGT  
1321 -----+-----+-----+-----+-----+-----+ 1380  
AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA  
  
L G R C K R D D F V E G L S F A R S G G -  
  
AACTGGGCTGAATGTTTCGCTTAA  
1381 -----+-----+----- 1404  
TTGACCCGACTTACAAAGCGAATT  
  
N W A E C F A \*

Fig. 22c

48/56

```
ATGGGCGTGTTCGTGCTGCTACTGTCCATTGCCACCTTGTTTCGGTTCACATCCGGTACC
1  -----+-----+-----+-----+-----+-----+ 60
TACCCGCACAAGCAGCACGATGACAGGTAACGGTGAACAAGCCAAGGTGTAGGCCATGG

M G V F V V L L S I A T L F G S T S G T -

GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
61  -----+-----+-----+-----+-----+-----+ 120
CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACCTGCCACCAATGGTTACA

A L G P R G N S H S C D T V D G G Y Q C -

TTCCCAGAAATTTCTCACTTGTGGGGTACCTACTCTCCATACTTCTCTTTGGCAGACGAA
121 -----+-----+-----+-----+-----+-----+ 180
AAGGGTCTTTAAAGAGTGAACACCCCATGGATGAGAGGTATGAAGAGAAACCGTCTGCTT

F P E I S H L W G T Y S P Y F S L A D E -

TCTGCTATTTCTCCAGACGTCCCAAAGGACTGTAGAGTTACTTTCGTTCAAGTTTTGTCT
181 -----+-----+-----+-----+-----+-----+ 240
AGACGATAAAGAGGTCTGCAGGGTTTCCTGACATCTCAATGAAAGCAAGTTCAAAACAGA

S A I S P D V P K D C R V T F V Q V L S -

AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA
241 -----+-----+-----+-----+-----+-----+ 300
TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAAACTAACTT

R H G A R Y P T S S A S K A Y S A L I E -

GCTATTCAAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAC
301 -----+-----+-----+-----+-----+-----+ 360
CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTG

A I Q K N A T A F K G K Y A F L K T Y N -

TACACTTTGGGTGCTGACGACTTGACTCCATTTCGGTGAAAACCAAATGGTTAACTCTGGT
361 -----+-----+-----+-----+-----+-----+ 420
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTTTGGTTTACCAATTGAGACCA

Y T L G A D D L T P F G E N Q M V N S G -

ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT
421 -----+-----+-----+-----+-----+-----+ 480
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

I K F Y R R Y K A L A R K I V P F I R A -

TCTGGTTCTGACAGAGTTATTGCTTCTGCTGAAAAGTTCATTGAAGGTTTCCAATCTGCT
481 -----+-----+-----+-----+-----+-----+ 540
AGACCAAGACTGTCTCAATAACGAAGACGACTTTTCAAGTAACTTCCAAGGTTAGACGA

S G S D R V I A S A E K F I E G F Q S A -
```

Fig. 23a

49/56

AAGTTGGCTGACCCAGGTTCTCAACCACACCAAGCTTCTCCAGTTATTAACGTGATCATT  
541 -----+-----+-----+-----+-----+-----+ 600  
TTCAACCGACTGGGTCCAAGAGTTGGTGTGGTTCGAAGAGGTCAATAATTGCACTAGTAA  
K L A D P G S Q P H Q A S P V I N V I I -  
CCAGAAGGATCCGGTTACAACAACACTTTGGACCATGGTCTTTGTACTGCTTTCGAAGAC  
601 -----+-----+-----+-----+-----+-----+ 660  
GGTCTTCCTAGGCCAATGTTGTTGTGAAACCTGGTACCAGAAACATGACGAAAGCTTCTG  
P E G S G Y N N T L D H G L C T A F E D -  
TCTACCCTAGGTGACGACGTTGAAGCTAACTTCACTGCTTTGTTTCGCTCCAGCTATTAGA  
661 -----+-----+-----+-----+-----+-----+ 720  
AGATGGGATCCACTGCTGCAACTTCGATTGAAGTGACGAAACAAGCGAGGTGATAATCT  
S T L G D D V E A N F T A L F A P A I R -  
GCTAGATTGGAAGCTGACTTGCCAGGTGTTACTTTGACTGACGAAGACGTTGTTTACTTG  
721 -----+-----+-----+-----+-----+-----+ 780  
CGATCTAACCTTCGACTGAACGGTCCACAATGAACTGACTGCTTCTGCAACAAATGAAC  
A R L E A D L P G V T L T D E D V V Y L -  
ATGGACATGTGTCCATTCGACACTGTCGCTAGAACTTCTGACGCTACTGAATTGTCTCCA  
781 -----+-----+-----+-----+-----+-----+ 840  
TACCTGTACACAGGTAAGCTGTGACAGCGATCTTGAAGACTGCGATGACTTAACAGAGGT  
M D M C P F D T V A R T S D A T E L S P -  
TTCTGTGCTTTGTTCACTCACGACGAATGGATCCAATACGACTACTTGCAAAGCTTGGGT  
841 -----+-----+-----+-----+-----+-----+ 900  
AAGACACGAAACAAGTGAGTGCTGCTTACCTAGGTTATGCTGATGAACGTTTCGAACCCA  
F C A L F T H D E W I Q Y D Y L Q S L G -  
AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGCT  
901 -----+-----+-----+-----+-----+-----+ 960  
TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCGA  
K Y Y G Y G A G N P L G P A Q G V G F A -  
AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC  
961 -----+-----+-----+-----+-----+-----+ 1020  
TTGCTTAACCTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG  
N E L I A R L T H S P V Q D H T S T N H -  
ACTTTGGACTCTAACCCAGCTACTTTCCCATTTGAACGCTACTTTGTACGCTGACTTCTCT  
1021 -----+-----+-----+-----+-----+-----+ 1080  
TGAAACCTGAGATTGGGTGCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA  
T L D S N P A T F P L N A T L Y A D F S -

Fig. 23b

50/56

CACGACAACACTATGATATCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACCAAGCCA  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GTGCTGTTGTGATACTATAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGGTTCGGT  
H D N T M I S I F F A L G L Y N G T K P -  
TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGGACT  
1141 -----+-----+-----+-----+-----+-----+ 1200  
AACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA  
L S T T S V E S I E E T D G Y S A S W T -  
GTTCCATTTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTCAAGCTGAAAAGGAACCA  
1201 -----+-----+-----+-----+-----+-----+ 1260  
CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACAGTTCGACTTTTCCTTGGT  
V P F A A R A Y V E M M Q C Q A E K E P -  
TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG  
1261 -----+-----+-----+-----+-----+-----+ 1320  
AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACCTGTTTC  
L V R V L V N D R V V P L H G C A V D K -  
TTGGGTTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT  
1321 -----+-----+-----+-----+-----+-----+ 1380  
AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA  
L G R C K R D D F V E G L S F A R S G G -  
AACTGGGCTGAATGTTTCGCTTAA  
1381 -----+-----+----- 1404  
TTGACCCGACTTACAAAGCGAATT  
N W A E C F A \*

Fig. 23c

51/56

```
ATGGGCGTGTTTCGTCGTGCTACTGTCCATTGCCACCTTGTTTCGGTTCACATCCGGTACC
1  -----+-----+-----+-----+-----+-----+ 60
TACCCGCACAAGCAGCACGATGACAGGTAACGGTGGAACAAGCCAAGGTGTAGGCCATGG

M G V F V V L L S I A T L F G S T S G T -

GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
61  -----+-----+-----+-----+-----+-----+ 120
CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACTGCCACCAATGGTTACA

A L G P R G N S H S C D T V D G G Y Q C -

TTCCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTTGGCTGACGAA
121 -----+-----+-----+-----+-----+-----+ 180
AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT

F P E I S H L W G T Y S P F F S L A D E -

TCTGCTATTTCTCCAGACGTTCCAAAGGGTTGTAGAGTTACTTTCGTTCAAGTTTTGTCT
181 -----+-----+-----+-----+-----+-----+ 240
AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA

S A I S P D V P K G C R V T F V Q V L S -

AGACACGGTGCTAGATACCCAACTTCTTCTAAGTCTAAGGCTTACTCTGCTTTGATTGAA
241 -----+-----+-----+-----+-----+-----+ 300
TCTGTGCCACGATCTATGGGTTGAAGAAGATTCAGATTCCGAATGAGACGAACTAACTT

R H G A R Y P T S S K S K A Y S A L I E -

GCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAT
301 -----+-----+-----+-----+-----+-----+ 360
CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTA

A I Q K N A T A F K G K Y A F L K T Y N -

TACACTTTGGGTGCTGACGACTTGACTCCATTTCGGTGAACAACAAATGGTTAACTCTGGT
361 -----+-----+-----+-----+-----+-----+ 420
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTGTTTACCAATTGAGACCA

Y T L G A D D L T P F G E Q Q M V N S G -

ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTCATTAGAGCT
421 -----+-----+-----+-----+-----+-----+ 480
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

I K F Y R R Y K A L A R K I V P F I R A -

TCTGGTTCTGACAGAGTTATTGCTTCTGCCGAAAAGTTCATTGAAGGTTTCCAATCTGCT
481 -----+-----+-----+-----+-----+-----+ 540
AGACCAAGACTGTCTCAATAACGAAGACGGCTTTTCAAGTAACTTCCAAAGGTTAGACGA

S G S D R V I A S A E K F I E G F Q S A -
```

Fig. 24a

52/56

AAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTAACGTTATTATT  
541 -----+-----+-----+-----+-----+-----+ 600  
TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCTGAAGAGGTCAATAATTGCAATAATAA  
K L A D P G A N P H Q A S P V I N V I I -  
CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTCGAAGAA  
601 -----+-----+-----+-----+-----+-----+ 660  
GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT  
P E G A G Y N N T L D H G L C T A F E E -  
TCTACCCTAGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTTCGCTCCACCAATTAGA  
661 -----+-----+-----+-----+-----+-----+ 720  
AGATGGGATCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT  
S T L G D D V E A N F T A V F A P P I R -  
GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACGTTGTAACTTG  
721 -----+-----+-----+-----+-----+-----+ 780  
CGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGCAACAATTGAAC  
A R L E A H L P G V N L T D E D V V N L -  
ATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTCAATTGTCTCCA  
781 -----+-----+-----+-----+-----+-----+ 840  
TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT  
M D M C P F D T V A R T S D A T Q L S P -  
TTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT  
841 -----+-----+-----+-----+-----+-----+ 900  
AAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA  
F C D L F T H D E W I Q Y D Y L Q S L G -  
AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT  
901 -----+-----+-----+-----+-----+-----+ 960  
TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTCGAGTTCCACAACCAAAGCAA  
K Y Y G Y G A G N P L G P A Q G V G F V -  
AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC  
961 -----+-----+-----+-----+-----+-----+ 1020  
TTGCTTAACTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG  
N E L I A R L T H S P V Q D H T S T N H -  
ACTTTGGACTCTAACCCAGCTACTTTCCCATTTGAACGCTACTTTGTACGCTGACTTCTCT  
1021 -----+-----+-----+-----+-----+-----+ 1080  
TGAAACCTGAGATTGGGTGCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA  
T L D S N P A T F P L N A T L Y A D F S -

Fig. 24b

53/56

CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTCCGGT  
  
H D N T M V S I F F A L G L Y N G T K P -  
  
TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGGACT  
1141 -----+-----+-----+-----+-----+-----+ 1200  
AACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA  
  
L S T T S V E S I E E T D G Y S A S W T -  
  
GTTCCATTGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA  
1201 -----+-----+-----+-----+-----+-----+ 1260  
CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT  
  
V P F A A R A Y V E M M Q C E A E K E P -  
  
TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG  
1261 -----+-----+-----+-----+-----+-----+ 1320  
AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACCTGTTT  
  
L V R V L V N D R V V P L H G C A V D K -  
  
TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTCGCTAGATCTGGTGGT  
1321 -----+-----+-----+-----+-----+-----+ 1380  
AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA  
  
L G R C K R D D F V E G L S F A R S G G -  
  
AACTGGGAAGAATGTTTCGCTTAA  
1381 -----+-----+----- 1404  
TTGACCCTTCTTACAAAGCGAATT  
  
N W E E C F A \*

Fig. 24c

54/56

```
ATGGGCGTGTTTCGTGCTGCTACTGTCCATTGCCACCTTGTTTCGGTTCACATCCGGTACC
1  -----+-----+-----+-----+-----+-----+ 60
TACCCGCACAAGCAGCACGATGACAGGTAACGGTGAACAAGCCAAGGTGTAGGCCATGG

M G V F V V L L S I A T L F G S T S G T -

GCCTTGGGTCCTCGTGGTAATTCTCACTCTTGTGACACTGTTGACGGTGGTTACCAATGT
61  -----+-----+-----+-----+-----+-----+ 120
CGGAACCCAGGAGCACCATTAAGAGTGAGAACACTGTGACAACCTGCCACCAATGGTTACA

A L G P R G N S H S C D T V D G G Y Q C -

TTCCCAGAAATTTCTCACTTGTGGGGTACATACTCTCCATTCTTCTCTTTGGCTGACGAA
121 -----+-----+-----+-----+-----+-----+ 180
AAGGGTCTTTAAAGAGTGAACACCCCATGTATGAGAGGTAAGAAGAGAAACCGACTGCTT

F P E I S H L W G T Y S P F F S L A D E -

TCTGCTATTTCTCCAGACGTTCCAAAGGGTGTAGAGTTACTTTCGTTCAAGTTTTGTCT
181 -----+-----+-----+-----+-----+-----+ 240
AGACGATAAAGAGGTCTGCAAGGTTTCCCAACATCTCAATGAAAGCAAGTTCAAAACAGA

S A I S P D V P K G C R V T F V Q V L S -

AGACACGGTGCTAGATACCCAACTTCTTCTGCGTCTAAGGCTTACTCTGCTTTGATTGAA
241 -----+-----+-----+-----+-----+-----+ 300
TCTGTGCCACGATCTATGGGTTGAAGAAGACGCAGATTCCGAATGAGACGAACTAACTT

R H G A R Y P T S S A S K A Y S A L I E -

GCTATTCAAAGAACGCTACTGCTTTCAAGGGTAAGTACGCTTTCTTGAAGACTTACAAT
301 -----+-----+-----+-----+-----+-----+ 360
CGATAAGTTTTCTTGCGATGACGAAAGTTCCCATTCATGCGAAAGAACTTCTGAATGTTA

A I Q K N A T A F K G K Y A F L K T Y N -

TACACTTTGGGTGCTGACGACTTGACTCCATTTCGGTGAACAACAAATGGTTAACTCTGGT
361 -----+-----+-----+-----+-----+-----+ 420
ATGTGAAACCCACGACTGCTGAACTGAGGTAAGCCACTTGTTGTTTACCAATTGAGACCA

Y T L G A D D L T P F G E Q Q M V N S G -

ATTAAGTTCTACAGAAGATACAAGGCTTTGGCTAGAAAGATTGTTCCATTTCATTAGAGCT
421 -----+-----+-----+-----+-----+-----+ 480
TAATTCAAGATGTCTTCTATGTTCCGAAACCGATCTTTCTAACAAGGTAAGTAATCTCGA

I K F Y R R Y K A L A R K I V P F I R A -

TCTGGTTCTGACAGAGTTATTGCTTCTGCCGAAAAGTTCATTGAAGGTTTCCAATCTGCT
481 -----+-----+-----+-----+-----+-----+ 540
AGACCAAGACTGTCTCAATAACGAAGACGGCTTTTCAAGTAACTTCCAAAGGTTAGACGA

S G S D R V I A S A E K F I E G F Q S A -
```

Fig. 25a

55/56

AAGTTGGCTGACCCAGGTGCTAACCCACACCAAGCTTCTCCAGTTATTAACGTTATTATT  
541 -----+-----+-----+-----+-----+-----+ 600  
TTCAACCGACTGGGTCCACGATTGGGTGTGGTTCGAAGAGGTCAATAATTGCAATAATAA  
K L A D P G A N P H Q A S P V I N V I I -  
CCAGAAGGTGCTGGTTACAACAACACTTTGGACCACGGTTTGTGTACTGCTTTCGAAGAA  
601 -----+-----+-----+-----+-----+ 660  
GGTCTTCCACGACCAATGTTGTTGTGAAACCTGGTGCCAAACACATGACGAAAGCTTCTT  
P E G A G Y N N T L D H G L C T A F E E -  
TCTACCCTAGGTGACGACGTTGAAGCTAACTTCACTGCTGTTTTTCGCTCCACCAATTAGA  
661 -----+-----+-----+-----+-----+ 720  
AGATGGGATCCACTGCTGCAACTTCGATTGAAGTGACGACAAAAGCGAGGTGGTTAATCT  
S T L G D D V E A N F T A V F A P P I R -  
GCTAGATTGGAAGCTCACTTGCCAGGTGTTAACTTGACTGACGAAGACGTTGTAACTTG  
721 -----+-----+-----+-----+-----+ 780  
CGATCTAACCTTCGAGTGAACGGTCCACAATTGAACTGACTGCTTCTGCAACAATTGAAC  
A R L E A H L P G V N L T D E D V V N L -  
ATGGACATGTGTCCATTCGACACTGTTGCTAGAACTTCTGACGCTACTCAATTGTCTCCA  
781 -----+-----+-----+-----+-----+ 840  
TACCTGTACACAGGTAAGCTGTGACAACGATCTTGAAGACTGCGATGAGTTAACAGAGGT  
M D M C P F D T V A R T S D A T Q L S P -  
TTCTGTGACTTGTTCACTCACGACGAATGGATTCAATACGACTACTTGCAATCTTTGGGT  
841 -----+-----+-----+-----+-----+ 900  
AAGACACTGAACAAGTGAGTGCTGCTTACCTAAGTTATGCTGATGAACGTTAGAAACCCA  
F C D L F T H D E W I Q Y D Y L Q S L G -  
AAGTACTACGGTTACGGTGCTGGTAACCCATTGGGTCCAGCTCAAGGTGTTGGTTTCGTT  
901 -----+-----+-----+-----+-----+ 960  
TTCATGATGCCAATGCCACGACCATTGGGTAACCCAGGTGAGTTCCACAACCAAAGCAA  
K Y Y G Y G A G N P L G P A Q G V G F V -  
AACGAATTGATTGCTAGATTGACTCACTCTCCAGTTCAAGACCACACTTCTACTAACCAC  
961 -----+-----+-----+-----+-----+ 1020  
TTGCTTAACCTAACGATCTAACTGAGTGAGAGGTCAAGTTCTGGTGTGAAGATGATTGGTG  
N E L I A R L T H S P V Q D H T S T N H -  
ACTTTGGACTCTAACCCAGCTACTTTCCCATTTGAACGCTACTTTGTACGCTGACTTCTCT  
1021 -----+-----+-----+-----+-----+ 1080  
TGAAACCTGAGATTGGGTGCGATGAAAGGGTAACTTGCGATGAAACATGCGACTGAAGAGA  
T L D S N P A T F P L N A T L Y A D F S -

Fig. 25b

56/56

CACGACAACACTATGGTTTCTATTTTCTTCGCTTTGGGTTTGTACAACGGTACTAAGCCA  
1081 -----+-----+-----+-----+-----+-----+ 1140  
GTGCTGTTGTGATACCAAAGATAAAAGAAGCGAAACCCAAACATGTTGCCATGATTTCGGT  
H D N T M V S I F F A L G L Y N G T K P -  
TTGTCTACTACTTCTGTTGAATCTATTGAAGAACTGACGGTTACTCTGCTTCTTGGACT  
1141 -----+-----+-----+-----+-----+-----+ 1200  
AACAGATGATGAAGACAACCTTAGATAACTTCTTTGACTGCCAATGAGACGAAGAACCTGA  
L S T T S V E S I E E T D G Y S A S W T -  
GTTCCATTCGCTGCTAGAGCTTACGTTGAAATGATGCAATGTGAAGCTGAAAAGGAACCA  
1201 -----+-----+-----+-----+-----+-----+ 1260  
CAAGGTAAGCGACGATCTCGAATGCAACTTTACTACGTTACACTTCGACTTTTCCTTGGT  
V P F A A R A Y V E M M Q C E A E K E P -  
TTGGTTAGAGTTTTGGTTAACGACAGAGTTGTTCCATTGCACGGTTGTGCTGTTGACAAG  
1261 -----+-----+-----+-----+-----+-----+ 1320  
AACCAATCTCAAAACCAATTGCTGTCTCAACAAGGTAACGTGCCAACACGACAACCTGTTT  
L V R V L V N D R V V P L H G C A V D K -  
TTGGGTAGATGTAAGAGAGACGACTTCGTTGAAGGTTTGTCTTTTCGCTAGATCTGGTGGT  
1321 -----+-----+-----+-----+-----+-----+ 1380  
AACCCATCTACATTCTCTCTGCTGAAGCAACTTCCAAACAGAAAGCGATCTAGACCACCA  
L G R C K R D D F V E G L S F A R S G G -  
AACTGGGAAGAATGTTTCGCTTAA  
1381 -----+-----+----- 1404  
TTGACCCTTCTTACAAAGCGAATT  
N W E E C F A \*

Fig. 25c

## SEQUENCE LISTING

&lt;110&gt; Novo Nordisk A/S

&lt;120&gt; Improved phytases

&lt;130&gt; seqlist171299

&lt;140&gt;

&lt;141&gt;

&lt;150&gt; DK 99/00092

&lt;151&gt; 1999-01-22

&lt;150&gt; DK 99/01340

&lt;151&gt; 1999-09-21

&lt;160&gt; 89

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 440

&lt;212&gt; PRT

&lt;213&gt; Aspergillus terreus 9A-1

&lt;400&gt; 1

Lys His Ser Asp Cys Asn Ser Val Asp His Gly Tyr Gln Cys Phe Pro  
1 5 10 15

Glu Leu Ser His Lys Trp Gly Leu Tyr Ala Pro Tyr Phe Ser Leu Gln  
20 25 30

Asp Glu Ser Pro Phe Pro Leu Asp Val Pro Glu Asp Cys His Ile Thr  
35 40 45

Phe Val Gln Val Leu Ala Arg His Gly Ala Arg Ser Pro Thr His Ser  
50 55 60

Lys Thr Lys Ala Tyr Ala Ala Thr Ile Ala Ala Ile Gln Lys Ser Ala  
65 70 75 80

Thr Ala Phe Pro Gly Lys Tyr Ala Phe Leu Gln Ser Tyr Asn Tyr Ser  
85 90 95

Leu Asp Ser Glu Glu Leu Thr Pro Phe Gly Arg Asn Gln Leu Arg Asp  
100 105 110

Leu Gly Ala Gln Phe Tyr Glu Arg Tyr Asn Ala Leu Thr Arg His Ile  
115 120 125

Asn Pro Phe Val Arg Ala Thr Asp Ala Ser Arg Val His Glu Ser Ala  
130 135 140

## 2

Glu Lys Phe Val Glu Gly Phe Gln Thr Ala Arg Gln Asp Asp His His  
 145 150 155 160  
 Ala Asn Pro His Gln Pro Ser Pro Arg Val Asp Val Ala Ile Pro Glu  
 165 170 175  
 Gly Ser Ala Tyr Asn Asn Thr Leu Glu His Ser Leu Cys Thr Ala Phe  
 180 185 190  
 Glu Ser Ser Thr Val Gly Asp Asp Ala Val Ala Asn Phe Thr Ala Val  
 195 200 205  
 Phe Ala Pro Ala Ile Ala Gln Arg Leu Glu Ala Asp Leu Pro Gly Val  
 210 215 220  
 Gln Leu Ser Thr Asp Asp Val Val Asn Leu Met Ala Met Cys Pro Phe  
 225 230 235 240  
 Glu Thr Val Ser Leu Thr Asp Asp Ala His Thr Leu Ser Pro Phe Cys  
 245 250 255  
 Asp Leu Phe Thr Ala Thr Glu Trp Thr Gln Tyr Asn Tyr Leu Leu Ser  
 260 265 270  
 Leu Asp Lys Tyr Tyr Gly Tyr Gly Gly Gly Asn Pro Leu Gly Pro Val  
 275 280 285  
 Gln Gly Val Gly Trp Ala Asn Glu Leu Met Ala Arg Leu Thr Arg Ala  
 290 295 300  
 Pro Val His Asp His Thr Cys Val Asn Asn Thr Leu Asp Ala Ser Pro  
 305 310 315 320  
 Ala Thr Phe Pro Leu Asn Ala Thr Leu Tyr Ala Asp Phe Ser His Asp  
 325 330 335  
 Ser Asn Leu Val Ser Ile Phe Trp Ala Leu Gly Leu Tyr Asn Gly Thr  
 340 345 350  
 Ala Pro Leu Ser Gln Thr Ser Val Glu Ser Val Ser Gln Thr Asp Gly  
 355 360 365  
 Tyr Ala Ala Ala Trp Thr Val Pro Phe Ala Ala Arg Ala Tyr Val Glu  
 370 375 380  
 Met Met Gln Cys Arg Ala Glu Lys Glu Pro Leu Val Arg Val Leu Val  
 385 390 395 400  
 Asn Asp Arg Val Met Pro Leu His Gly Cys Pro Thr Asp Lys Leu Gly  
 405 410 415  
 Arg Cys Lys Arg Asp Ala Phe Val Ala Gly Leu Ser Phe Ala Gln Ala  
 420 425 430

Gly Gly Asn Trp Ala Asp Cys Phe  
 435 440

<210> 2

<211> 440

<212> PRT

<213> *Aspergillus terreus* cbs

<400> 2

Asn His Ser Asp Cys Thr Ser Val Asp Arg Gly Tyr Gln Cys Phe Pro  
 1 5 10 15

Glu Leu Ser His Lys Trp Gly Leu Tyr Ala Pro Tyr Phe Ser Leu Gln  
 20 25 30

Asp Glu Ser Pro Phe Pro Leu Asp Val Pro Asp Asp Cys His Ile Thr  
 35 40 45

Phe Val Gln Val Leu Ala Arg His Gly Ala Arg Ser Pro Thr Asp Ser  
 50 55 60

Lys Thr Lys Ala Tyr Ala Ala Thr Ile Ala Ala Ile Gln Lys Asn Ala  
 65 70 75 80

Thr Ala Leu Pro Gly Lys Tyr Ala Phe Leu Lys Ser Tyr Asn Tyr Ser  
 85 90 95

Met Gly Ser Glu Asn Leu Thr Pro Phe Gly Arg Asn Gln Leu Gln Asp  
 100 105 110

Leu Gly Ala Gln Phe Tyr Arg Arg Tyr Asp Thr Leu Thr Arg His Ile  
 115 120 125

Asn Pro Phe Val Arg Ala Ala Asp Ser Ser Arg Val His Glu Ser Ala  
 130 135 140

Glu Lys Phe Val Glu Gly Phe Gln Asn Ala Arg Gln Gly Asp Pro His  
 145 150 155 160

Ala Asn Pro His Gln Pro Ser Pro Arg Val Asp Val Val Ile Pro Glu  
 165 170 175

Gly Thr Ala Tyr Asn Asn Thr Leu Glu His Ser Ile Cys Thr Ala Phe  
 180 185 190

Glu Ala Ser Thr Val Gly Asp Ala Ala Ala Asp Asn Phe Thr Ala Val  
 195 200 205

Phe Ala Pro Ala Ile Ala Lys Arg Leu Glu Ala Asp Leu Pro Gly Val  
 210 215 220

Gln Leu Ser Ala Asp Asp Val Val Asn Leu Met Ala Met Cys Pro Phe  
 225 230 235 240

Glu	Thr	Val	Ser	Leu	Thr	Asp	Asp	Ala	His	Thr	Leu	Ser	Pro	Phe	Cys
				245					250					255	
Asp	Leu	Phe	Thr	Ala	Ala	Glu	Trp	Thr	Gln	Tyr	Asn	Tyr	Leu	Leu	Ser
			260					265					270		
Leu	Asp	Lys	Tyr	Tyr	Gly	Tyr	Gly	Gly	Gly	Asn	Pro	Leu	Gly	Pro	Val
		275					280					285			
Gln	Gly	Val	Gly	Trp	Ala	Asn	Glu	Leu	Ile	Ala	Arg	Leu	Thr	Arg	Ser
	290					295					300				
Pro	Val	His	Asp	His	Thr	Cys	Val	Asn	Asn	Thr	Leu	Asp	Ala	Asn	Pro
305					310					315					320
Ala	Thr	Phe	Pro	Leu	Asn	Ala	Thr	Leu	Tyr	Ala	Asp	Phe	Ser	His	Asp
				325					330					335	
Ser	Asn	Leu	Val	Ser	Ile	Phe	Trp	Ala	Leu	Gly	Leu	Tyr	Asn	Gly	Thr
			340					345					350		
Lys	Pro	Leu	Ser	Gln	Thr	Thr	Val	Glu	Asp	Ile	Thr	Arg	Thr	Asp	Gly
		355					360					365			
Tyr	Ala	Ala	Ala	Trp	Thr	Val	Pro	Phe	Ala	Ala	Arg	Ala	Tyr	Ile	Glu
	370					375					380				
Met	Met	Gln	Cys	Arg	Ala	Glu	Lys	Gln	Pro	Leu	Val	Arg	Val	Leu	Val
385					390					395					400
Asn	Asp	Arg	Val	Met	Pro	Leu	His	Gly	Cys	Ala	Val	Asp	Asn	Leu	Gly
				405					410					415	
Arg	Cys	Lys	Arg	Asp	Asp	Phe	Val	Glu	Gly	Leu	Ser	Phe	Ala	Arg	Ala
			420					425					430		
Gly	Gly	Asn	Trp	Ala	Glu	Cys	Phe								
		435					440								

Asn	Glu	Ser	Ala	Ile	Ser	Pro	Asp	Val	Pro	Ala	Gly	Cys	Arg	Val	Thr	
	35						40					45				
Phe	Ala	Gln	Val	Leu	Ser	Arg	His	Gly	Ala	Arg	Tyr	Pro	Thr	Glu	Ser	
	50					55					60					
Lys	Gly	Lys	Lys	Tyr	Ser	Ala	Leu	Ile	Glu	Glu	Ile	Gln	Gln	Asn	Val	
	65				70					75					80	
Thr	Thr	Phe	Asp	Gly	Lys	Tyr	Ala	Phe	Leu	Lys	Thr	Tyr	Asn	Tyr	Ser	
				85					90					95		
Leu	Gly	Ala	Asp	Asp	Leu	Thr	Pro	Phe	Gly	Glu	Gln	Glu	Leu	Val	Asn	
			100					105					110			
Ser	Gly	Ile	Lys	Phe	Tyr	Gln	Arg	Tyr	Glu	Ser	Leu	Thr	Arg	Asn	Ile	
		115					120					125				
Ile	Pro	Phe	Ile	Arg	Ser	Ser	Gly	Ser	Ser	Arg	Val	Ile	Ala	Ser	Gly	
	130					135					140					
Glu	Lys	Phe	Ile	Glu	Gly	Phe	Gln	Ser	Thr	Lys	Leu	Lys	Asp	Pro	Arg	
	145				150					155					160	
Ala	Gln	Pro	Gly	Gln	Ser	Ser	Pro	Lys	Ile	Asp	Val	Val	Ile	Ser	Glu	
				165					170					175		
Ala	Ser	Ser	Ser	Asn	Asn	Thr	Leu	Asp	Pro	Gly	Thr	Cys	Thr	Val	Phe	
			180					185					190			
Glu	Asp	Ser	Glu	Leu	Ala	Asp	Thr	Val	Glu	Ala	Asn	Phe	Thr	Ala	Thr	
		195					200					205				
Phe	Ala	Pro	Ser	Ile	Arg	Gln	Arg	Leu	Glu	Asn	Asp	Leu	Ser	Gly	Val	
	210					215					220					
Thr	Leu	Thr	Asp	Thr	Glu	Val	Thr	Tyr	Leu	Met	Asp	Met	Cys	Ser	Phe	
	225				230					235					240	
Asp	Thr	Ile	Ser	Thr	Ser	Thr	Val	Asp	Thr	Lys	Leu	Ser	Pro	Phe	Cys	
				245					250					255		
Asp	Leu	Phe	Thr	His	Asp	Glu	Trp	Ile	His	Tyr	Asp	Tyr	Leu	Gln	Ser	
			260					265					270			
Leu	Lys	Lys	Tyr	Tyr	Gly	His	Gly	Ala	Gly	Asn	Pro	Leu	Gly	Pro	Thr	
		275					280					285				
Gln	Gly	Val	Gly	Tyr	Ala	Asn	Glu	Leu	Ile	Ala	Arg	Leu	Thr	His	Ser	
	290					295					300					
Pro	Val	His	Asp	Asp	Thr	Ser	Ser	Asn	His	Thr	Leu	Asp	Ser	Asn	Pro	
	305				310					315					320	

6

Ala Thr Phe Pro Leu Asn Ser Thr Leu Tyr Ala Asp Phe Ser His Asp  
 325 330 335

Asn Gly Ile Ile Ser Ile Leu Phe Ala Leu Gly Leu Tyr Asn Gly Thr  
 340 345 350

Lys Pro Leu Ser Thr Thr Thr Val Glu Asn Ile Thr Gln Thr Asp Gly  
 355 360 365

Phe Ser Ser Ala Trp Thr Val Pro Phe Ala Ser Arg Leu Tyr Val Glu  
 370 375 380

Met Met Gln Cys Gln Ala Glu Gln Glu Pro Leu Val Arg Val Leu Val  
 385 390 395 400

Asn Asp Arg Val Val Pro Leu His Gly Cys Pro Ile Asp Ala Leu Gly  
 405 410 415

Arg Cys Thr Arg Asp Ser Phe Val Arg Gly Leu Ser Phe Ala Arg Ser  
 420 425 430

Gly Gly Asp Trp Ala Glu Cys Ser Ala  
 435 440

&lt;210&gt; 4

&lt;211&gt; 441

&lt;212&gt; PRT

&lt;213&gt; Aspergillus niger T213

&lt;400&gt; 4

Asn Gln Ser Ser Cys Asp Thr Val Asp Gln Gly Tyr Gln Cys Phe Ser  
 1 5 10 15

Glu Thr Ser His Leu Trp Gly Gln Tyr Ala Pro Phe Phe Ser Leu Ala  
 20 25 30

Asn Glu Ser Val Ile Ser Pro Asp Val Pro Ala Gly Cys Arg Val Thr  
 35 40 45

Phe Ala Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Glu Ser  
 50 55 60

Lys Gly Lys Lys Tyr Ser Ala Leu Ile Glu Glu Ile Gln Gln Asn Val  
 65 70 75 80

Thr Thr Phe Asp Gly Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Ser  
 85 90 95

Leu Gly Ala Asp Asp Leu Thr Pro Phe Gly Glu Gln Glu Leu Val Asn  
 100 105 110

Ser Gly Ile Lys Phe Tyr Gln Arg Tyr Glu Ser Leu Thr Arg Asn Ile  
 115 120 125

Ile Pro Phe Ile Arg Ser Ser Gly Ser Ser Arg Val Ile Ala Ser Gly  
 130 135 140  
 Glu Lys Phe Ile Glu Gly Phe Gln Ser Thr Lys Leu Lys Asp Pro Arg  
 145 150 155 160  
 Ala Gln Pro Gly Gln Ser Ser Pro Lys Ile Asp Val Val Ile Ser Glu  
 165 170 175  
 Ala Ser Ser Ser Asn Asn Thr Leu Asp Pro Gly Thr Cys Thr Val Phe  
 180 185 190  
 Glu Asp Ser Glu Leu Ala Asp Thr Val Glu Ala Asn Phe Thr Ala Thr  
 195 200 205  
 Phe Ala Pro Ser Ile Arg Gln Arg Leu Glu Asn Asp Leu Ser Gly Val  
 210 215 220  
 Thr Leu Thr Asp Thr Glu Val Thr Tyr Leu Met Asp Met Cys Ser Phe  
 225 230 235 240  
 Asp Thr Ile Ser Thr Ser Thr Val Asp Thr Lys Leu Ser Pro Phe Cys  
 245 250 255  
 Asp Leu Phe Thr His Asp Glu Trp Ile His Tyr Asp Tyr Leu Arg Ser  
 260 265 270  
 Leu Lys Lys Tyr Tyr Gly His Gly Ala Gly Asn Pro Leu Gly Pro Thr  
 275 280 285  
 Gln Gly Val Gly Tyr Ala Asn Glu Leu Ile Ala Arg Leu Thr His Ser  
 290 295 300  
 Pro Val His Asp Asp Thr Ser Ser Asn His Thr Leu Asp Ser Asn Pro  
 305 310 315 320  
 Ala Thr Phe Pro Leu Asn Ser Thr Leu Tyr Ala Asp Phe Ser His Asp  
 325 330 335  
 Asn Gly Ile Ile Ser Ile Leu Phe Ala Leu Gly Leu Tyr Asn Gly Thr  
 340 345 350  
 Lys Pro Leu Ser Thr Thr Thr Val Glu Asn Ile Thr Gln Thr Asp Gly  
 355 360 365  
 Phe Ser Ser Ala Trp Thr Val Pro Phe Ala Ser Arg Leu Tyr Val Glu  
 370 375 380  
 Met Met Gln Cys Gln Ala Glu Gln Glu Pro Leu Val Arg Val Leu Val  
 385 390 395 400  
 Asn Asp Arg Val Val Pro Leu His Gly Cys Pro Ile Asp Ala Leu Gly  
 405 410 415

Arg Cys Thr Arg Asp Ser Phe Val Arg Gly Leu Ser Phe Ala Arg Ser  
 420 425 430

Gly Gly Asp Trp Ala Glu Cys Phe Ala  
 435 440

<210> 5

<211> 441

<212> PRT

<213> Aspergillus niger NRRL3135

<400> 5

Asn Gln Ser Ser Cys Asp Thr Val Asp Gln Gly Tyr Gln Cys Phe Ser  
 1 5 10 15

Glu Thr Ser His Leu Trp Gly Gln Tyr Ala Pro Phe Phe Ser Leu Ala  
 20 25 30

Asn Glu Ser Val Ile Ser Pro Glu Val Pro Ala Gly Cys Arg Val Thr  
 35 40 45

Phe Ala Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Asp Ser  
 50 55 60

Lys Gly Lys Lys Tyr Ser Ala Leu Ile Glu Glu Ile Gln Gln Asn Ala  
 65 70 75 80

Thr Thr Phe Asp Gly Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Ser  
 85 90 95

Leu Gly Ala Asp Asp Leu Thr Pro Phe Gly Glu Gln Glu Leu Val Asn  
 100 105 110

Ser Gly Ile Lys Phe Tyr Gln Arg Tyr Glu Ser Leu Thr Arg Asn Ile  
 115 120 125

Val Pro Phe Ile Arg Ser Ser Gly Ser Ser Arg Val Ile Ala Ser Gly  
 130 135 140

Lys Lys Phe Ile Glu Gly Phe Gln Ser Thr Lys Leu Lys Asp Pro Arg  
 145 150 155 160

Ala Gln Pro Gly Gln Ser Ser Pro Lys Ile Asp Val Val Ile Ser Glu  
 165 170 175

Ala Ser Ser Ser Asn Asn Thr Leu Asp Pro Gly Thr Cys Thr Val Phe  
 180 185 190

Glu Asp Ser Glu Leu Ala Asp Thr Val Glu Ala Asn Phe Thr Ala Thr  
 195 200 205

## 9

Phe Val Pro Ser Ile Arg Gln Arg Leu Glu Asn Asp Leu Ser Gly Val  
 210 215 220  
 Thr Leu Thr Asp Thr Glu Val Thr Tyr Leu Met Asp Met Cys Ser Phe  
 225 230 235 240  
 Asp Thr Ile Ser Thr Ser Thr Val Asp Thr Lys Leu Ser Pro Phe Cys  
 245 250 255  
 Asp Leu Phe Thr His Asp Glu Trp Ile Asn Tyr Asp Tyr Leu Gln Ser  
 260 265 270  
 Leu Lys Lys Tyr Tyr Gly His Gly Ala Gly Asn Pro Leu Gly Pro Thr  
 275 280 285  
 Gln Gly Val Gly Tyr Ala Asn Glu Leu Ile Ala Arg Leu Thr His Ser  
 290 295 300  
 Pro Val His Asp Asp Thr Ser Ser Asn His Thr Leu Asp Ser Ser Pro  
 305 310 315 320  
 Ala Thr Phe Pro Leu Asn Ser Thr Leu Tyr Ala Asp Phe Ser His Asp  
 325 330 335  
 Asn Gly Ile Ile Ser Ile Leu Phe Ala Leu Gly Leu Tyr Asn Gly Thr  
 340 345 350  
 Lys Pro Leu Ser Thr Thr Thr Val Glu Asn Ile Thr Gln Thr Asp Gly  
 355 360 365  
 Phe Ser Ser Ala Trp Thr Val Pro Phe Ala Ser Arg Leu Tyr Val Glu  
 370 375 380  
 Met Met Gln Cys Gln Ala Glu Gln Glu Pro Leu Val Arg Val Leu Val  
 385 390 395 400  
 Asn Asp Arg Val Val Pro Leu His Gly Cys Pro Val Asp Ala Leu Gly  
 405 410 415  
 Arg Cys Thr Arg Asp Ser Phe Val Arg Gly Leu Ser Phe Ala Arg Ser  
 420 425 430  
 Gly Gly Asp Trp Ala Glu Cys Phe Ala  
 435 440

&lt;210&gt; 6

&lt;211&gt; 440

&lt;212&gt; PRT

&lt;213&gt; Aspergillus fumigatus 13073

&lt;400&gt; 6

Gly Ser Lys Ser Cys Asp Thr Val Asp Leu Gly Tyr Gln Cys Ser Pro  
 1 5 10 15

Ala Thr Ser His Leu Trp Gly Gln Tyr Ser Pro Phe Phe Ser Leu Glu  
                   20                  25                  30  
 Asp Glu Leu Ser Val Ser Ser Lys Leu Pro Lys Asp Cys Arg Ile Thr  
           35                  40                  45  
 Leu Val Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser  
       50                  55                  60  
 Lys Ser Lys Lys Tyr Lys Lys Leu Val Thr Ala Ile Gln Ala Asn Ala  
   65                  70                  75                  80  
 Thr Asp Phe Lys Gly Lys Phe Ala Phe Leu Lys Thr Tyr Asn Tyr Thr  
                   85                  90                  95  
 Leu Gly Ala Asp Asp Leu Phe Gln Gln Ala Lys Leu Ala Asp Pro Gly  
                   100                  105                  110  
 Ala Thr Asn Arg Ala Ala Pro Ala Ile Ser Val Ile Ile Pro Glu Ser  
           115                  120                  125  
 Glu Thr Phe Asn Asn Thr Leu Asp His Gly Val Cys Thr Lys Phe Glu  
       130                  135                  140  
 Ala Ser Gln Leu Thr Pro Phe Gly Glu Gln Gln Leu Val Asn Ser Gly  
   145                  150                  155                  160  
 Ile Lys Phe Tyr Gln Arg Tyr Lys Ala Leu Ala Arg Ser Val Val Pro  
                   165                  170                  175  
 Phe Ile Arg Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Gly Glu Lys  
           180                  185                  190  
 Phe Ile Glu Gly Gly Asp Glu Val Ala Ala Asn Phe Thr Ala Leu Phe  
           195                  200                  205  
 Ala Pro Asp Ile Arg Ala Arg Ala Glu Lys His Leu Pro Gly Val Thr  
       210                  215                  220  
 Leu Thr Asp Glu Asp Val Val Ser Leu Met Asp Met Cys Ser Phe Asp  
   225                  230                  235                  240  
 Thr Val Ala Arg Thr Ser Asp Ala Ser Gln Leu Ser Pro Phe Cys Gln  
                   245                  250                  255  
 Leu Phe Thr His Asn Glu Trp Lys Lys Tyr Asn Tyr Leu Gln Ser Leu  
           260                  265                  270  
 Gly Lys Tyr Tyr Gly Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln  
       275                  280                  285  
 Gly Ile Gly Phe Thr Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro  
       290                  295                  300

Val Gln Asp His Thr Ser Thr Asn Ser Thr Leu Val Ser Asn Pro Ala  
 305 310 315 320  
 Thr Phe Pro Leu Asn Ala Thr Met Tyr Val Asp Phe Ser His Asp Asn  
 325 330 335  
 Ser Met Val Ser Ile Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Glu  
 340 345 350  
 Pro Leu Ser Arg Thr Ser Val Glu Ser Ala Lys Glu Leu Asp Gly Tyr  
 355 360 365  
 Ser Ala Ser Trp Val Val Pro Phe Gly Ala Arg Ala Tyr Phe Glu Thr  
 370 375 380  
 Met Gln Cys Lys Ser Glu Lys Glu Pro Leu Val Arg Ala Leu Ile Asn  
 385 390 395 400  
 Asp Arg Val Val Pro Leu His Gly Cys Asp Val Asp Lys Leu Gly Arg  
 405 410 415  
 Cys Lys Leu Asn Asp Phe Val Lys Gly Leu Ser Trp Ala Arg Ser Gly  
 420 425 430  
 Gly Asn Trp Gly Glu Cys Phe Ser  
 435 440

&lt;210&gt; 7

&lt;211&gt; 440

&lt;212&gt; PRT

&lt;213&gt; Aspergillus fumigatus 32722

&lt;400&gt; 7

Gly Ser Lys Ser Cys Asp Thr Val Asp Leu Gly Tyr Gln Cys Ser Pro  
 1 5 10 15  
 Ala Thr Ser His Leu Trp Gly Gln Tyr Ser Pro Phe Phe Ser Leu Glu  
 20 25 30  
 Asp Glu Leu Ser Val Ser Ser Lys Leu Pro Lys Asp Cys Arg Ile Thr  
 35 40 45  
 Leu Val Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser  
 50 55 60  
 Lys Ser Lys Lys Tyr Lys Lys Leu Val Thr Ala Ile Gln Ala Asn Ala  
 65 70 75 80  
 Thr Asp Phe Lys Gly Lys Phe Ala Phe Leu Lys Thr Tyr Asn Tyr Thr  
 85 90 95

## 12

Leu Gly Ala Asp Asp Leu Thr Pro Phe Gly Glu Gln Gln Leu Val Asn  
 100 105 110  
 Ser Gly Ile Lys Phe Tyr Gln Arg Tyr Lys Ala Leu Ala Arg Ser Val  
 115 120 125  
 Val Pro Phe Ile Arg Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Gly  
 130 135 140  
 Glu Lys Phe Ile Glu Gly Phe Gln Gln Ala Lys Leu Ala Asp Pro Gly  
 145 150 155 160  
 Ala Thr Asn Arg Ala Ala Pro Ala Ile Ser Val Ile Ile Pro Glu Ser  
 165 170 175  
 Glu Thr Phe Asn Asn Thr Leu Asp His Gly Val Cys Thr Lys Phe Glu  
 180 185 190  
 Ala Ser Gln Leu Gly Asp Glu Val Ala Ala Asn Phe Thr Ala Leu Phe  
 195 200 205  
 Ala Pro Asp Ile Arg Ala Arg Ala Glu Lys His Leu Pro Gly Val Thr  
 210 215 220  
 Leu Thr Asp Glu Asp Val Val Ser Leu Met Asp Met Cys Ser Phe Asp  
 225 230 235 240  
 Thr Val Ala Arg Thr Ser Asp Ala Ser Gln Leu Ser Pro Phe Cys Gln  
 245 250 255  
 Leu Phe Thr His Asn Glu Trp Lys Lys Tyr Asn Tyr Leu Gln Ser Leu  
 260 265 270  
 Gly Lys Tyr Tyr Gly Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln  
 275 280 285  
 Gly Ile Gly Phe Thr Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro  
 290 295 300  
 Val Gln Asp His Thr Ser Thr Asn Ser Thr Leu Val Ser Asn Pro Ala  
 305 310 315 320  
 Thr Phe Pro Leu Asn Ala Thr Met Tyr Val Asp Phe Ser His Asp Asn  
 325 330 335  
 Ser Met Val Ser Ile Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Gly  
 340 345 350  
 Pro Leu Ser Arg Thr Ser Val Glu Ser Ala Lys Glu Leu Asp Gly Tyr  
 355 360 365  
 Ser Ala Ser Trp Val Val Pro Phe Gly Ala Arg Ala Tyr Phe Glu Thr  
 370 375 380

13

Met Gln Cys Lys Ser Glu Lys Glu Pro Leu Val Arg Ala Leu Ile Asn  
 385 390 395 400

Asp Arg Val Val Pro Leu His Gly Cys Asp Val Asp Lys Leu Gly Arg  
 405 410 415

Cys Lys Leu Asn Asp Phe Val Lys Gly Leu Ser Trp Ala Arg Ser Gly  
 420 425 430

Gly Asn Trp Gly Glu Cys Phe Ser  
 435 440

&lt;210&gt; 8

&lt;211&gt; 440

&lt;212&gt; PRT

<213> *Aspergillus fumigatus* 58128

&lt;400&gt; 8

Gly Ser Lys Ser Cys Asp Thr Val Asp Leu Gly Tyr Gln Cys Ser Pro  
 1 5 10 15

Ala Thr Ser His Leu Trp Gly Gln Tyr Ser Pro Phe Phe Ser Leu Glu  
 20 25 30

Asp Glu Leu Ser Val Ser Ser Lys Leu Pro Lys Asp Cys Arg Ile Thr  
 35 40 45

Leu Val Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser  
 50 55 60

Lys Ser Lys Lys Tyr Lys Lys Leu Val Thr Ala Ile Gln Ala Asn Ala  
 65 70 75 80

Thr Asp Phe Lys Gly Lys Phe Ala Phe Leu Lys Thr Tyr Asn Tyr Thr  
 85 90 95

Leu Gly Ala Asp Asp Leu Thr Pro Phe Gly Glu Gln Gln Leu Val Asn  
 100 105 110

Ser Gly Ile Lys Phe Tyr Gln Arg Tyr Lys Ala Leu Ala Arg Ser Val  
 115 120 125

Val Pro Phe Ile Arg Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Gly  
 130 135 140

Glu Lys Phe Ile Glu Gly Phe Gln Gln Ala Lys Leu Ala Asp Pro Gly  
 145 150 155 160

Ala Thr Asn Arg Ala Ala Pro Ala Ile Ser Val Ile Ile Pro Glu Ser  
 165 170 175

Glu Thr Phe Asn Asn Thr Leu Asp His Gly Val Cys Thr Lys Phe Glu  
 180 185 190

14

Ala Ser Gln Leu Gly Asp Glu Val Ala Ala Asn Phe Thr Ala Leu Phe  
 195 200 205  
 Ala Pro Asp Ile Arg Ala Arg Ala Glu Lys His Leu Pro Gly Val Thr  
 210 215 220  
 Leu Thr Asp Glu Asp Val Val Ser Leu Met Asp Met Cys Ser Phe Asp  
 225 230 235 240  
 Thr Val Ala Arg Thr Ser Asp Ala Ser Gln Leu Ser Pro Phe Cys Gln  
 245 250 255  
 Leu Phe Thr His Asn Glu Trp Lys Lys Tyr Asn Tyr Leu Gln Ser Leu  
 260 265 270  
 Gly Lys Tyr Tyr Gly Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln  
 275 280 285  
 Gly Ile Gly Phe Thr Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro  
 290 295 300  
 Val Gln Asp His Thr Ser Thr Asn Ser Thr Leu Val Ser Asn Pro Ala  
 305 310 315 320  
 Thr Phe Pro Leu Asn Ala Thr Met Tyr Val Asp Phe Ser His Asp Asn  
 325 330 335  
 Ser Met Val Ser Ile Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Glu  
 340 345 350  
 Pro Leu Ser Arg Thr Ser Val Glu Ser Ala Lys Glu Leu Asp Gly Tyr  
 355 360 365  
 Ser Ala Ser Trp Val Val Pro Phe Gly Ala Arg Ala Tyr Phe Glu Thr  
 370 375 380  
 Met Gln Cys Lys Ser Glu Lys Glu Ser Leu Val Arg Ala Leu Ile Asn  
 385 390 395 400  
 Asp Arg Val Val Pro Leu His Gly Cys Asp Val Asp Lys Leu Gly Arg  
 405 410 415  
 Cys Lys Leu Asn Asp Phe Val Lys Gly Leu Ser Trp Ala Arg Ser Gly  
 420 425 430  
 Gly Asn Trp Gly Glu Cys Phe Ser  
 435 440

&lt;210&gt; 9

&lt;211&gt; 440

&lt;212&gt; PRT

&lt;213&gt; Aspergillus fumigatus 26906

15

&lt;400&gt; 9

Gly	Ser	Lys	Ser	Cys	Asp	Thr	Val	Asp	Leu	Gly	Tyr	Gln	Cys	Ser	Pro	1	5	10	15
Ala	Thr	Ser	His	Leu	Trp	Gly	Gln	Tyr	Ser	Pro	Phe	Phe	Ser	Leu	Glu	20	25	30	
Asp	Glu	Leu	Ser	Val	Ser	Ser	Lys	Leu	Pro	Lys	Asp	Cys	Arg	Ile	Thr	35	40	45	
Leu	Val	Gln	Val	Leu	Ser	Arg	His	Gly	Ala	Arg	Tyr	Pro	Thr	Ser	Ser	50	55	60	
Lys	Ser	Lys	Lys	Tyr	Lys	Lys	Leu	Val	Thr	Ala	Ile	Gln	Ala	Asn	Ala	65	70	75	80
Thr	Asp	Phe	Lys	Gly	Lys	Phe	Ala	Phe	Leu	Lys	Thr	Tyr	Asn	Tyr	Thr	85	90	95	
Leu	Gly	Ala	Asp	Asp	Leu	Thr	Ala	Phe	Gly	Glu	Gln	Gln	Leu	Val	Asn	100	105	110	
Ser	Gly	Ile	Lys	Phe	Tyr	Gln	Arg	Tyr	Lys	Ala	Leu	Ala	Arg	Ser	Val	115	120	125	
Val	Pro	Phe	Ile	Arg	Ala	Ser	Gly	Ser	Asp	Arg	Val	Ile	Ala	Ser	Gly	130	135	140	
Glu	Lys	Phe	Ile	Glu	Gly	Phe	Gln	Gln	Ala	Lys	Leu	Ala	Asp	Pro	Gly	145	150	155	160
Ala	Thr	Asn	Arg	Ala	Ala	Pro	Ala	Ile	Ser	Val	Ile	Ile	Pro	Glu	Ser	165	170	175	
Glu	Thr	Phe	Asn	Asn	Thr	Leu	Asp	His	Gly	Val	Cys	Thr	Lys	Phe	Glu	180	185	190	
Ala	Ser	Gln	Leu	Gly	Asp	Glu	Val	Ala	Ala	Asn	Phe	Thr	Ala	Leu	Phe	195	200	205	
Ala	Pro	Asp	Ile	Arg	Ala	Arg	Ala	Lys	Lys	His	Leu	Pro	Gly	Val	Thr	210	215	220	
Leu	Thr	Asp	Glu	Asp	Val	Val	Ser	Leu	Met	Asp	Met	Cys	Ser	Phe	Asp	225	230	235	240
Thr	Val	Ala	Arg	Thr	Ser	Asp	Ala	Ser	Gln	Leu	Ser	Pro	Phe	Cys	Gln	245	250	255	
Leu	Phe	Thr	His	Asn	Glu	Trp	Lys	Lys	Tyr	Asn	Tyr	Leu	Gln	Ser	Leu	260	265	270	

16

Gly Lys Tyr Tyr Gly Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln  
 275 280 285  
 Gly Ile Gly Phe Thr Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro  
 290 295 300  
 Val Gln Asp His Thr Ser Thr Asn Ser Thr Leu Val Ser Asn Pro Ala  
 305 310 315 320  
 Thr Phe Pro Leu Asn Ala Thr Met Tyr Val Asp Phe Ser His Asp Asn  
 325 330 335  
 Ser Met Val Ser Ile Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Glu  
 340 345 350  
 Pro Leu Ser Arg Thr Ser Val Glu Ser Ala Lys Glu Leu Asp Gly Tyr  
 355 360 365  
 Ser Ala Ser Trp Val Val Pro Phe Gly Ala Arg Ala Tyr Phe Glu Thr  
 370 375 380  
 Met Gln Cys Lys Ser Glu Lys Glu Pro Leu Val Arg Ala Leu Ile Asn  
 385 390 395 400  
 Asp Arg Val Val Pro Leu His Gly Cys Asp Val Asp Lys Leu Gly Arg  
 405 410 415  
 Cys Lys Leu Asn Asp Phe Val Lys Gly Leu Ser Trp Ala Arg Ser Gly  
 420 425 430  
 Gly Asn Trp Gly Glu Cys Phe Ser  
 435 440

&lt;210&gt; 10

&lt;211&gt; 440

&lt;212&gt; PRT

&lt;213&gt; Aspergillus fumigatus 32239

&lt;400&gt; 10

Gly Ser Lys Ala Cys Asp Thr Val Glu Leu Gly Tyr Gln Cys Ser Pro  
 1 5 10 15  
 Gly Thr Ser His Leu Trp Gly Gln Tyr Ser Pro Phe Phe Ser Leu Glu  
 20 25 30  
 Asp Glu Leu Ser Val Ser Ser Asp Leu Pro Lys Asp Cys Arg Val Thr  
 35 40 45  
 Phe Val Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ala Ser  
 50 55 60  
 Lys Ser Lys Lys Tyr Lys Lys Leu Val Thr Ala Ile Gln Lys Asn Ala  
 65 70 75 80

Thr Glu Phe Lys Gly Lys Phe Ala Phe Leu Glu Thr Tyr Asn Tyr Thr  
                                     85                                    90                                    95  
 Leu Gly Ala Asp Asp Leu Thr Pro Phe Gly Glu Gln Gln Met Val Asn  
                                     100                                    105                                    110  
 Ser Gly Ile Lys Phe Tyr Gln Lys Tyr Lys Ala Leu Ala Gly Ser Val  
                                     115                                    120                                    125  
 Val Pro Phe Ile Arg Ser Ser Gly Ser Asp Arg Val Ile Ala Ser Gly  
                                     130                                    135                                    140  
 Glu Lys Phe Ile Glu Gly Phe Gln Gln Ala Asn Val Ala Asp Pro Gly  
                                     145                                    150                                    155                                    160  
 Ala Thr Asn Arg Ala Ala Pro Val Ile Ser Val Ile Ile Pro Glu Ser  
                                     165                                    170                                    175  
 Glu Thr Tyr Asn Asn Thr Leu Asp His Ser Val Cys Thr Asn Phe Glu  
                                     180                                    185                                    190  
 Ala Ser Glu Leu Gly Asp Glu Val Glu Ala Asn Phe Thr Ala Leu Phe  
                                     195                                    200                                    205  
 Ala Pro Ala Ile Arg Ala Arg Ile Glu Lys His Leu Pro Gly Val Gln  
                                     210                                    215                                    220  
 Leu Thr Asp Asp Asp Val Val Ser Leu Met Asp Met Cys Ser Phe Asp  
                                     225                                    230                                    235                                    240  
 Thr Val Ala Arg Thr Ala Asp Ala Ser Glu Leu Ser Pro Phe Cys Ala  
                                     245                                    250                                    255  
 Ile Phe Thr His Asn Glu Trp Lys Lys Tyr Asp Tyr Leu Gln Ser Leu  
                                     260                                    265                                    270  
 Gly Lys Tyr Tyr Gly Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln  
                                     275                                    280                                    285  
 Gly Ile Gly Phe Thr Asn Glu Leu Ile Ala Arg Leu Thr Asn Ser Pro  
                                     290                                    295                                    300  
 Val Gln Asp His Thr Ser Thr Asn Ser Thr Leu Asp Ser Asp Pro Ala  
                                     305                                    310                                    315                                    320  
 Thr Phe Pro Leu Asn Ala Thr Ile Tyr Val Asp Phe Ser His Asp Asn  
                                     325                                    330                                    335  
 Gly Met Ile Pro Ile Phe Phe Ala Met Gly Leu Tyr Asn Gly Thr Glu  
                                     340                                    345                                    350  
 Pro Leu Ser Gln Thr Ser Glu Glu Ser Thr Lys Glu Ser Asn Gly Tyr  
                                     355                                    360                                    365

Ser Ala Ser Trp Ala Val Pro Phe Gly Ala Arg Ala Tyr Phe Glu Thr  
 370 375 380

Met Gln Cys Lys Ser Glu Lys Glu Pro Leu Val Arg Ala Leu Ile Asn  
 385 390 395 400

Asp Arg Val Val Pro Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg  
 405 410 415

Cys Lys Leu Lys Asp Phe Val Lys Gly Leu Ser Trp Ala Arg Ser Gly  
 420 425 430

Gly Asn Ser Glu Gln Ser Phe Ser  
 435 440

<210> 11  
 <211> 439  
 <212> PRT  
 <213> Emericella nidulans

<400> 11  
 Gln Asn His Ser Cys Asn Thr Ala Asp Gly Gly Tyr Gln Cys Phe Pro  
 1 5 10 15

Asn Val Ser His Val Trp Gly Gln Tyr Ser Pro Tyr Phe Ser Ile Glu  
 20 25 30

Gln Glu Ser Ala Ile Ser Glu Asp Val Pro His Gly Cys Glu Val Thr  
 35 40 45

Phe Val Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Glu Ser  
 50 55 60

Lys Ser Lys Ala Tyr Ser Gly Leu Ile Glu Ala Ile Gln Lys Asn Ala  
 65 70 75 80

Thr Ser Phe Trp Gly Gln Tyr Ala Phe Leu Glu Ser Tyr Asn Tyr Thr  
 85 90 95

Leu Gly Ala Asp Asp Leu Thr Ile Phe Gly Glu Asn Gln Met Val Asp  
 100 105 110

Ser Gly Ala Lys Phe Tyr Arg Arg Tyr Lys Asn Leu Ala Arg Lys Asn  
 115 120 125

Thr Pro Phe Ile Arg Ala Ser Gly Ser Asp Arg Val Val Ala Ser Ala  
 130 135 140

Glu Lys Phe Ile Asn Gly Phe Arg Lys Ala Gln Leu His Asp His Gly  
 145 150 155 160

19

Ser Gly Gln Ala Thr Pro Val Val Asn Val Ile Ile Pro Glu Ile Asp  
 165 170 175  
 Gly Phe Asn Asn Thr Leu Asp His Ser Thr Cys Val Ser Phe Glu Asn  
 180 185 190  
 Asp Glu Arg Ala Asp Glu Ile Glu Ala Asn Phe Thr Ala Ile Met Gly  
 195 200 205  
 Pro Pro Ile Arg Lys Arg Leu Glu Asn Asp Leu Pro Gly Ile Lys Leu  
 210 215 220  
 Thr Asn Glu Asn Val Ile Tyr Leu Met Asp Met Cys Ser Phe Asp Thr  
 225 230 235 240  
 Met Ala Arg Thr Ala His Gly Thr Glu Leu Ser Pro Phe Cys Ala Ile  
 245 250 255  
 Phe Thr Glu Lys Glu Trp Leu Gln Tyr Asp Tyr Leu Gln Ser Leu Ser  
 260 265 270  
 Lys Tyr Tyr Gly Tyr Gly Ala Gly Ser Pro Leu Gly Pro Ala Gln Gly  
 275 280 285  
 Ile Gly Phe Thr Asn Glu Leu Ile Ala Arg Leu Thr Gln Ser Pro Val  
 290 295 300  
 Gln Asp Asn Thr Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr  
 305 310 315 320  
 Phe Pro Leu Asp Arg Lys Leu Tyr Ala Asp Phe Ser His Asp Asn Ser  
 325 330 335  
 Met Ile Ser Ile Phe Phe Ala Met Gly Leu Tyr Asn Gly Thr Gln Pro  
 340 345 350  
 Leu Ser Met Asp Ser Val Glu Ser Ile Gln Glu Met Asp Gly Tyr Ala  
 355 360 365  
 Ala Ser Trp Thr Val Pro Phe Gly Ala Arg Ala Tyr Phe Glu Leu Met  
 370 375 380  
 Gln Cys Glu Lys Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg  
 385 390 395 400  
 Val Val Pro Leu His Gly Cys Ala Val Asp Lys Phe Gly Arg Cys Thr  
 405 410 415  
 Leu Asp Asp Trp Val Glu Gly Leu Asn Phe Ala Arg Ser Gly Gly Asn  
 420 425 430  
 Trp Lys Thr Cys Phe Thr Leu  
 435

20

<210> 12  
 <211> 443  
 <212> PRT  
 <213> Talaromyces thermophilus

<400> 12

Asp	Ser	His	Ser	Cys	Asn	Thr	Val	Glu	Gly	Gly	Tyr	Gln	Cys	Arg	Pro
1				5					10					15	
Glu	Ile	Ser	His	Ser	Trp	Gly	Gln	Tyr	Ser	Pro	Phe	Phe	Ser	Leu	Ala
			20					25					30		
Asp	Gln	Ser	Glu	Ile	Ser	Pro	Asp	Val	Pro	Gln	Asn	Cys	Lys	Ile	Thr
		35					40					45			
Phe	Val	Gln	Leu	Leu	Ser	Arg	His	Gly	Ala	Arg	Tyr	Pro	Thr	Ser	Ser
	50					55					60				
Lys	Thr	Glu	Leu	Tyr	Ser	Gln	Leu	Ile	Ser	Arg	Ile	Gln	Lys	Thr	Ala
65					70					75					80
Thr	Ala	Tyr	Lys	Gly	Tyr	Tyr	Ala	Phe	Leu	Lys	Asp	Tyr	Arg	Tyr	Gln
				85					90					95	
Leu	Gly	Ala	Asn	Asp	Leu	Thr	Pro	Phe	Gly	Glu	Asn	Gln	Met	Ile	Gln
			100					105					110		
Leu	Gly	Ile	Lys	Phe	Tyr	Asn	His	Tyr	Lys	Ser	Leu	Ala	Arg	Asn	Ala
		115					120						125		
Val	Pro	Phe	Val	Arg	Cys	Ser	Gly	Ser	Asp	Arg	Val	Ile	Ala	Ser	Gly
	130					135					140				
Arg	Leu	Phe	Ile	Glu	Gly	Phe	Gln	Ser	Ala	Lys	Val	Leu	Asp	Pro	His
145					150					155					160
Ser	Asp	Lys	His	Asp	Ala	Pro	Pro	Thr	Ile	Asn	Val	Ile	Ile	Glu	Glu
				165					170					175	
Gly	Pro	Ser	Tyr	Asn	Asn	Thr	Leu	Asp	Thr	Gly	Ser	Cys	Pro	Val	Phe
			180					185					190		
Glu	Asp	Ser	Ser	Gly	Gly	His	Asp	Ala	Gln	Glu	Lys	Phe	Ala	Lys	Gln
	195						200					205			
Phe	Ala	Pro	Ala	Ile	Leu	Glu	Lys	Ile	Lys	Asp	His	Leu	Pro	Gly	Val
	210					215					220				
Asp	Leu	Ala	Val	Ser	Asp	Val	Pro	Tyr	Leu	Met	Asp	Leu	Cys	Pro	Phe
225					230					235					240
Glu	Thr	Leu	Ala	Arg	Asn	His	Thr	Asp	Thr	Leu	Ser	Pro	Phe	Cys	Ala
			245						250					255	

21

Leu Ser Thr Gln Glu Glu Trp Gln Ala Tyr Asp Tyr Tyr Gln Ser Leu  
                   260                                  265                                  270  
 Gly Lys Tyr Tyr Gly Asn Gly Gly Gly Asn Pro Leu Gly Pro Ala Gln  
                   275                                  280                                  285  
 Gly Val Gly Phe Val Asn Glu Leu Ile Ala Arg Met Thr His Ser Pro  
                   290                                  295                                  300  
 Val Gln Asp Tyr Thr Thr Val Asn His Thr Leu Asp Ser Asn Pro Ala  
                   305                                  310                                  315                                  320  
 Thr Phe Pro Leu Asn Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn  
                   325                                  330                                  335  
 Thr Met Thr Ser Ile Phe Ala Ala Leu Gly Leu Tyr Asn Gly Thr Ala  
                   340                                  345                                  350  
 Lys Leu Ser Thr Thr Glu Ile Lys Ser Ile Glu Glu Thr Asp Gly Tyr  
                   355                                  360                                  365  
 Ser Ala Ala Trp Thr Val Pro Phe Gly Gly Arg Ala Tyr Ile Glu Met  
                   370                                  375                                  380  
 Met Gln Cys Asp Asp Ser Asp Glu Pro Val Val Arg Val Leu Val Asn  
                   385                                  390                                  395                                  400  
 Asp Arg Val Val Pro Leu His Gly Cys Glu Val Asp Ser Leu Gly Arg  
                   405                                  410                                  415  
 Cys Lys Arg Asp Asp Phe Val Arg Gly Leu Ser Phe Ala Arg Gln Gly  
                   420                                  425                                  430  
 Gly Asn Trp Glu Gly Cys Tyr Ala Ala Ser Glu  
                   435                                  440

&lt;210&gt; 13

&lt;211&gt; 466

&lt;212&gt; PRT

&lt;213&gt; Myceliophthora thermophila

&lt;400&gt; 13

Glu Ser Arg Pro Cys Asp Thr Pro Asp Leu Gly Phe Gln Cys Gly Thr  
   1                                  5                                  10                                  15  
 Ala Ile Ser His Phe Trp Gly Gln Tyr Ser Pro Tyr Phe Ser Val Pro  
                   20                                  25                                  30  
 Ser Glu Leu Asp Ala Ser Ile Pro Asp Asp Cys Glu Val Thr Phe Ala  
                   35                                  40                                  45

Gln	Val	Leu	Ser	Arg	His	Gly	Ala	Arg	Ala	Pro	Thr	Leu	Lys	Arg	Ala
50						55			60						
Ala	Ser	Tyr	Val	Asp	Leu	Ile	Asp	Arg	Ile	His	His	Gly	Ala	Ile	Ser
65			70						75			80			
Tyr	Gly	Pro	Gly	Tyr	Glu	Phe	Leu	Arg	Thr	Tyr	Asp	Tyr	Thr	Leu	Gly
			85						90			95			
Ala	Asp	Glu	Leu	Thr	Arg	Thr	Gly	Gln	Gln	Gln	Met	Val	Asn	Ser	Gly
			100			105						110			
Ile	Lys	Phe	Tyr	Arg	Arg	Tyr	Arg	Ala	Leu	Ala	Arg	Lys	Ser	Ile	Pro
115						120						125			
Phe	Val	Arg	Thr	Ala	Gly	Gln	Asp	Arg	Val	Val	His	Ser	Ala	Glu	Asn
130						135			140						
Phe	Thr	Gln	Gly	Phe	His	Ser	Ala	Leu	Leu	Ala	Asp	Arg	Gly	Ser	Thr
145			150						155			160			
Val	Arg	Pro	Thr	Leu	Pro	Tyr	Asp	Met	Val	Val	Ile	Pro	Glu	Thr	Ala
			165						170			175			
Gly	Ala	Asn	Asn	Thr	Leu	His	Asn	Asp	Leu	Cys	Thr	Ala	Phe	Glu	Glu
			180			185						190			
Gly	Pro	Tyr	Ser	Thr	Ile	Gly	Asp	Asp	Ala	Gln	Asp	Thr	Tyr	Leu	Ser
195						200						205			
Thr	Phe	Ala	Gly	Pro	Ile	Thr	Ala	Arg	Val	Asn	Ala	Asn	Leu	Pro	Gly
210						215			220						
Ala	Asn	Leu	Thr	Asp	Ala	Asp	Thr	Val	Ala	Leu	Met	Asp	Leu	Cys	Pro
225			230						235			240			
Phe	Glu	Thr	Val	Ala	Ser	Ser	Ser	Ser	Asp	Pro	Ala	Thr	Ala	Asp	Ala
			245						250			255			
Gly	Gly	Gly	Asn	Gly	Arg	Pro	Leu	Ser	Pro	Phe	Cys	Arg	Leu	Phe	Ser
			260			265						270			
Glu	Ser	Glu	Trp	Arg	Ala	Tyr	Asp	Tyr	Leu	Gln	Ser	Val	Gly	Lys	Trp
275						280						285			
Tyr	Gly	Tyr	Gly	Pro	Gly	Asn	Pro	Leu	Gly	Pro	Thr	Gln	Gly	Val	Gly
290						295			300						
Phe	Val	Asn	Glu	Leu	Leu	Ala	Arg	Leu	Ala	Gly	Val	Pro	Val	Arg	Asp
305			310						315			320			
Gly	Thr	Ser	Thr	Asn	Arg	Thr	Leu	Asp	Gly	Asp	Pro	Arg	Thr	Phe	Pro
			325						330			335			

23

Leu Gly Arg Pro Leu Tyr Ala Asp Phe Ser His Asp Asn Asp Met Met  
                   340                  345                  350  
 Gly Val Leu Gly Ala Leu Gly Ala Tyr Asp Gly Val Pro Pro Leu Asp  
                   355                  360                  365  
 Lys Thr Ala Arg Arg Asp Pro Glu Glu Leu Gly Gly Tyr Ala Ala Ser  
                   370                  375                  380  
 Trp Ala Val Pro Phe Ala Ala Arg Ile Tyr Val Glu Lys Met Arg Cys  
                   385                  390                  395                  400  
 Ser Gly Gly Gly Gly Gly Gly Gly Gly Glu Gly Arg Gln Glu Lys  
                   405                  410                  415  
 Asp Glu Glu Met Val Arg Val Leu Val Asn Asp Arg Val Met Thr Leu  
                   420                  425                  430  
 Lys Gly Cys Gly Ala Asp Glu Arg Gly Met Cys Thr Leu Glu Arg Phe  
                   435                  440                  445  
 Ile Glu Ser Met Ala Phe Ala Arg Gly Asn Gly Lys Trp Asp Leu Cys  
                   450                  455                  460  
 Phe Ala  
 465

&lt;210&gt; 14

&lt;211&gt; 441

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

 <223> Description of Artificial Sequence: consensus  
 phytase

&lt;400&gt; 14

Asn Ser His Ser Cys Asp Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro  
   1                  5                  10                  15  
 Glu Ile Ser His Leu Trp Gly Gln Tyr Ser Pro Tyr Phe Ser Leu Glu  
                   20                  25                  30  
 Asp Glu Ser Ala Ile Ser Pro Asp Val Pro Asp Asp Cys Arg Val Thr  
                   35                  40                  45  
 Phe Val Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser  
                   50                  55                  60  
 Lys Ser Lys Ala Tyr Ser Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala  
   65                  70                  75                  80

24

Thr	Ala	Phe	Lys	Gly	Lys	Tyr	Ala	Phe	Leu	Lys	Thr	Tyr	Asn	Tyr	Thr			
				85					90					95				
Leu	Gly	Ala	Asp	Asp	Leu	Thr	Pro	Phe	Gly	Glu	Asn	Gln	Met	Val	Asn			
			100					105					110					
Ser	Gly	Ile	Lys	Phe	Tyr	Arg	Arg	Tyr	Lys	Ala	Leu	Ala	Arg	Lys	Ile			
		115					120					125						
Val	Pro	Phe	Ile	Arg	Ala	Ser	Gly	Ser	Asp	Arg	Val	Ile	Ala	Ser	Ala			
	130					135					140							
Glu	Lys	Phe	Ile	Glu	Gly	Phe	Gln	Ser	Ala	Lys	Leu	Ala	Asp	Pro	Gly			
145					150					155					160			
Ser	Gln	Pro	His	Gln	Ala	Ser	Pro	Val	Ile	Asp	Val	Ile	Ile	Pro	Glu			
			165						170					175				
Gly	Ser	Gly	Tyr	Asn	Asn	Thr	Leu	Asp	His	Gly	Thr	Cys	Thr	Ala	Phe			
			180					185					190					
Glu	Asp	Ser	Glu	Leu	Gly	Asp	Asp	Val	Glu	Ala	Asn	Phe	Thr	Ala	Leu			
	195						200					205						
Phe	Ala	Pro	Ala	Ile	Arg	Ala	Arg	Leu	Glu	Ala	Asp	Leu	Pro	Gly	Val			
	210					215					220							
Thr	Leu	Thr	Asp	Glu	Asp	Val	Val	Tyr	Leu	Met	Asp	Met	Cys	Pro	Phe			
225					230					235					240			
Glu	Thr	Val	Ala	Arg	Thr	Ser	Asp	Ala	Thr	Glu	Leu	Ser	Pro	Phe	Cys			
			245						250					255				
Ala	Leu	Phe	Thr	His	Asp	Glu	Trp	Arg	Gln	Tyr	Asp	Tyr	Leu	Gln	Ser			
		260						265					270					
Leu	Gly	Lys	Tyr	Tyr	Gly	Tyr	Gly	Ala	Gly	Asn	Pro	Leu	Gly	Pro	Ala			
	275						280					285						
Gln	Gly	Val	Gly	Phe	Ala	Asn	Glu	Leu	Ile	Ala	Arg	Leu	Thr	Arg	Ser			
	290					295					300							
Pro	Val	Gln	Asp	His	Thr	Ser	Thr	Asn	His	Thr	Leu	Asp	Ser	Asn	Pro			
305					310					315				320				
Ala	Thr	Phe	Pro	Leu	Asn	Ala	Thr	Leu	Tyr	Ala	Asp	Phe	Ser	His	Asp			
			325						330					335				
Asn	Ser	Met	Ile	Ser	Ile	Phe	Phe	Ala	Leu	Gly	Leu	Tyr	Asn	Gly	Thr			
		340						345					350					
Ala	Pro	Leu	Ser	Thr	Thr	Ser	Val	Glu	Ser	Ile	Glu	Glu	Thr	Asp	Gly			
	355					360						365						

25

Tyr Ser Ala Ser Trp Thr Val Pro Phe Gly Ala Arg Ala Tyr Val Glu  
 370 375 380  
 Met Met Gln Cys Gln Ala Glu Lys Glu Pro Leu Val Arg Val Leu Val  
 385 390 395 400  
 Asn Asp Arg Val Val Pro Leu His Gly Cys Ala Val Asp Lys Leu Gly  
 405 410 415  
 Arg Cys Lys Arg Asp Asp Phe Val Glu Gly Leu Ser Phe Ala Arg Ser  
 420 425 430  
 Gly Gly Asn Trp Ala Glu Cys Phe Ala  
 435 440

<210> 15  
 <211> 1426  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: consensus  
 phytase

<220>  
 <221> CDS  
 <222> (12)..(1412)

<220>  
 <221> sig\_peptide  
 <222> (12)..(89)

<220>  
 <221> mat\_peptide  
 <222> (90)..(1412)

<400> 15  
 tatatgaatt c atg ggc gtg ttc gtc gtg cta ctg tcc att gcc acc ttg 50  
 Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu  
 -25 -20 -15  
 ttc ggt tcc aca tcc ggt acc gcc ttg ggt cct cgt ggt aat tct cac 98  
 Phe Gly Ser Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His  
 -10 -5 -1 1  
 tct tgt gac act gtt gac ggt ggt tac caa tgt ttc cca gaa att tct 146  
 Ser Cys Asp Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser  
 5 10 15  
 cac ttg tgg ggt caa tac tct cca tac ttc tct ttg gaa gac gaa tct 194  
 His Leu Trp Gly Gln Tyr Ser Pro Tyr Phe Ser Leu Glu Asp Glu Ser  
 20 25 30 35

26

gct att tct cca gac gtt cca gac gac tgt aga gtt act ttc gtt caa	242
Ala Ile Ser Pro Asp Val Pro Asp Asp Cys Arg Val Thr Phe Val Gln	
40 45 50	
ggt ttg tct aga cac ggt gct aga tac cca act tct tct aag tct aag	290
Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys	
55 60 65	
gct tac tct gct ttg att gaa gct att caa aag aac gct act gct ttc	338
Ala Tyr Ser Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe	
70 75 80	
aag ggt aag tac gct ttc ttg aag act tac aac tac act ttg ggt gct	386
Lys Gly Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala	
85 90 95	
gac gac ttg act cca ttc ggt gaa aac caa atg gtt aac tct ggt att	434
Asp Asp Leu Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile	
100 105 110 115	
aag ttc tac aga aga tac aag gct ttg gct aga aag att gtt cca ttc	482
Lys Phe Tyr Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe	
120 125 130	
att aga gct tct ggt tct gac aga gtt att gct tct gct gaa aag ttc	530
Ile Arg Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe	
135 140 145	
att gaa ggt ttc caa tct gct aag ttg gct gac cca ggt tct caa cca	578
Ile Glu Gly Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro	
150 155 160	
cac caa gct tct cca gtt att gac gtt att att cca gaa gga tcc ggt	626
His Gln Ala Ser Pro Val Ile Asp Val Ile Ile Pro Glu Gly Ser Gly	
165 170 175	
tac aac aac act ttg gac cac ggt act tgt act gct ttc gaa gac tct	674
Tyr Asn Asn Thr Leu Asp His Gly Thr Cys Thr Ala Phe Glu Asp Ser	
180 185 190 195	
gaa ttg ggt gac gac gtt gaa gct aac ttc act gct ttg ttc gct cca	722
Glu Leu Gly Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro	
200 205 210	
gct att aga gct aga ttg gaa gct gac ttg cca ggt gtt act ttg act	770
Ala Ile Arg Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr	
215 220 225	
gac gaa gac gtt gtt tac ttg atg gac atg tgt cca ttc gaa act gtt	818
Asp Glu Asp Val Val Tyr Leu Met Asp Met Cys Pro Phe Glu Thr Val	
230 235 240	
gct aga act tct gac gct act gaa ttg tct cca ttc tgt gct ttg ttc	866
Ala Arg Thr Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe	

27

245	250	255	
act cac gac gaa tgg aga caa tac gac tac ttg caa tct ttg ggt aag			914
Thr His Asp Glu Trp Arg Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys			
260	265	270	275
tac tac ggt tac ggt gct ggt aac cca ttg ggt cca gct caa ggt gtt			962
Tyr Tyr Gly Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val			
	280	285	290
ggt ttc gct aac gaa ttg att gct aga ttg act aga tct cca gtt caa			1010
Gly Phe Ala Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro Val Gln			
	295	300	305
gac cac act tct act aac cac act ttg gac tct aac cca gct act ttc			1058
Asp His Thr Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe			
	310	315	320
cca ttg aac gct act ttg tac gct gac ttc tct cac gac aac tct atg			1106
Pro Leu Asn Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Ser Met			
	325	330	335
att tct att ttc ttc gct ttg ggt ttg tac aac ggt act gct cca ttg			1154
Ile Ser Ile Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Ala Pro Leu			
	340	345	350
tct act act tct gtt gaa tct att gaa gaa act gac ggt tac tct gct			1202
Ser Thr Thr Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala			
	360	365	370
tct tgg act gtt cca ttc ggt gct aga gct tac gtt gaa atg atg caa			1250
Ser Trp Thr Val Pro Phe Gly Ala Arg Ala Tyr Val Glu Met Met Gln			
	375	380	385
tgt caa gct gaa aag gaa cca ttg gtt aga gtt ttg gtt aac gac aga			1298
Cys Gln Ala Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg			
	390	395	400
gtt gtt cca ttg cac ggt tgt gct gtt gac aag ttg ggt aga tgt aag			1346
Val Val Pro Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys			
	405	410	415
aga gac gac ttc gtt gaa ggt ttg tct ttc gct aga tct ggt ggt aac			1394
Arg Asp Asp Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn			
	420	425	430
tggt gct gaa tgt ttc gct taagaattca tata			1426
Trp Ala Glu Cys Phe Ala			
	440		

&lt;210&gt; 16

&lt;211&gt; 467

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

<223> Description of Artificial Sequence: consensus  
phytase

&lt;400&gt; 16

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Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser
-25                      -20                      -15

Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp
-10                      -5                      -1    1                      5

Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp
10                      15                      20

Gly Gln Tyr Ser Pro Tyr Phe Ser Leu Glu Asp Glu Ser Ala Ile Ser
25                      30                      35

Pro Asp Val Pro Asp Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser
40                      45                      50

Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Ala Tyr Ser
55                      60                      65                      70

Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys
75                      80                      85

Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu
90                      95                      100

Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile Lys Phe Tyr
105                      110                      115

Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala
120                      125                      130

Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly
135                      140                      145                      150

Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro His Gln Ala
155                      160                      165

Ser Pro Val Ile Asp Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn
170                      175                      180

Thr Leu Asp His Gly Thr Cys Thr Ala Phe Glu Asp Ser Glu Leu Gly
185                      190                      195

Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro Ala Ile Arg
200                      205                      210

Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr Asp Glu Asp
215                      220                      225                      230

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29

Val Val Tyr Leu Met Asp Met Cys Pro Phe Glu Thr Val Ala Arg Thr  
 235 240 245  
 Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe Thr His Asp  
 250 255 260  
 Glu Trp Arg Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly  
 265 270 275  
 Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Ala  
 280 285 290  
 Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro Val Gln Asp His Thr  
 295 300 305 310  
 Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn  
 315 320 325  
 Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Ser Met Ile Ser Ile  
 330 335 340  
 Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Ala Pro Leu Ser Thr Thr  
 345 350 355  
 Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr  
 360 365 370  
 Val Pro Phe Gly Ala Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala  
 375 380 385 390  
 Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
 395 400 405  
 Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
 410 415 420  
 Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu  
 425 430 435  
 Cys Phe Ala  
 440

&lt;210&gt; 17

&lt;211&gt; 422

&lt;212&gt; PRT

&lt;213&gt; Paxillus involutus phyA1

&lt;400&gt; 17

Ser Val Pro Lys Asn Thr Ala Pro Thr Phe Pro Ile Pro Glu Ser Glu  
 1 5 10 15

30

Gln Arg Asn Trp Ser Pro Tyr Ser Pro Tyr Phe Pro Leu Ala Glu Tyr  
 20 25 30  
 Lys Ala Pro Pro Ala Gly Cys Gln Ile Asn Gln Val Asn Ile Ile Gln  
 35 40 45  
 Arg His Gly Ala Arg Phe Pro Thr Ser Gly Ala Thr Thr Arg Ile Lys  
 50 55 60  
 Ala Gly Leu Thr Lys Leu Gln Gly Val Gln Asn Phe Thr Asp Ala Lys  
 65 70 75 80  
 Phe Asn Phe Ile Lys Ser Phe Lys Tyr Asp Leu Gly Asn Ser Asp Leu  
 85 90 95  
 Val Pro Phe Gly Ala Ala Gln Ser Phe Asp Ala Gly Gln Glu Ala Phe  
 100 105 110  
 Ala Arg Tyr Ser Lys Leu Val Ser Lys Asn Asn Leu Pro Phe Ile Arg  
 115 120 125  
 Ala Asp Gly Ser Asp Arg Val Val Asp Ser Ala Thr Asn Trp Thr Ala  
 130 135 140  
 Gly Phe Ala Ser Ala Ser His Asn Thr Val Gln Pro Lys Leu Asn Leu  
 145 150 155 160  
 Ile Leu Pro Gln Thr Gly Asn Asp Thr Leu Glu Asp Asn Met Cys Pro  
 165 170 175  
 Ala Ala Gly Asp Ser Asp Pro Gln Val Asn Ala Trp Leu Ala Val Ala  
 180 185 190  
 Phe Pro Ser Ile Thr Ala Arg Leu Asn Ala Ala Ala Pro Ser Val Asn  
 195 200 205  
 Leu Thr Asp Thr Asp Ala Phe Asn Leu Val Ser Leu Cys Ala Phe Leu  
 210 215 220  
 Thr Val Ser Lys Glu Lys Lys Ser Asp Phe Cys Thr Leu Phe Glu Gly  
 225 230 235 240  
 Ile Pro Gly Ser Phe Glu Ala Phe Ala Tyr Gly Gly Asp Leu Asp Lys  
 245 250 255  
 Phe Tyr Gly Thr Gly Tyr Gly Gln Glu Leu Gly Pro Val Gln Gly Val  
 260 265 270  
 Gly Tyr Val Asn Glu Leu Ile Ala Arg Leu Thr Asn Ser Ala Val Arg  
 275 280 285  
 Asp Asn Thr Gln Thr Asn Arg Thr Leu Asp Ala Ser Pro Val Thr Phe  
 290 295 300

31

Pro Leu Asn Lys Thr Phe Tyr Ala Asp Phe Ser His Asp Asn Leu Met  
 305 310 315 320  
 Val Ala Val Phe Ser Ala Met Gly Leu Phe Arg Gln Pro Ala Pro Leu  
 325 330 335  
 Ser Thr Ser Val Pro Asn Pro Trp Arg Thr Trp Arg Thr Ser Ser Leu  
 340 345 350  
 Val Pro Phe Ser Gly Arg Met Val Val Glu Arg Leu Ser Cys Phe Gly  
 355 360 365  
 Thr Thr Lys Val Arg Val Leu Val Gln Asp Gln Val Gln Pro Leu Glu  
 370 375 380  
 Phe Cys Gly Gly Asp Arg Asn Gly Leu Cys Thr Leu Ala Lys Phe Val  
 385 390 395 400  
 Glu Ser Gln Thr Phe Ala Arg Ser Asp Gly Ala Gly Asp Phe Glu Lys  
 405 410 415  
 Cys Phe Ala Thr Ser Ala  
 420

<210> 18  
 <211> 422  
 <212> PRT  
 <213> Paxillus involutus phyA2

<400> 18  
 Ser Val Pro Arg Asn Ile Ala Pro Lys Phe Ser Ile Pro Glu Ser Glu  
 1 5 10 15  
 Gln Arg Asn Trp Ser Pro Tyr Ser Pro Tyr Phe Pro Leu Ala Glu Tyr  
 20 25 30  
 Lys Ala Pro Pro Ala Gly Cys Glu Ile Asn Gln Val Asn Ile Ile Gln  
 35 40 45  
 Arg His Gly Ala Arg Phe Pro Thr Ser Gly Ala Ala Thr Arg Ile Lys  
 50 55 60  
 Ala Gly Leu Ser Lys Leu Gln Ser Val Gln Asn Phe Thr Asp Pro Lys  
 65 70 75 80  
 Phe Asp Phe Ile Lys Ser Phe Thr Tyr Asp Leu Gly Thr Ser Asp Leu  
 85 90 95  
 Val Pro Phe Gly Ala Ala Gln Ser Phe Asp Ala Gly Leu Glu Val Phe  
 100 105 110  
 Ala Arg Tyr Ser Lys Leu Val Ser Ser Asp Asn Leu Pro Phe Ile Arg  
 115 120 125

Ser Asp Gly Ser Asp Arg Val Val Asp Thr Ala Thr Asn Trp Thr Ala  
 130 135 140  
 Gly Phe Ala Ser Ala Ser Arg Asn Ala Ile Gln Pro Lys Leu Asp Leu  
 145 150 155 160  
 Ile Leu Pro Gln Thr Gly Asn Asp Thr Leu Glu Asp Asn Met Cys Pro  
 165 170 175  
 Ala Ala Gly Glu Ser Asp Pro Gln Val Asp Ala Trp Leu Ala Ser Ala  
 180 185 190  
 Phe Pro Ser Val Thr Ala Gln Leu Asn Ala Ala Ala Pro Gly Ala Asn  
 195 200 205  
 Leu Thr Asp Ala Asp Ala Phe Asn Leu Val Ser Leu Cys Pro Phe Met  
 210 215 220  
 Thr Val Ser Lys Glu Gln Lys Ser Asp Phe Cys Thr Leu Phe Glu Gly  
 225 230 235 240  
 Ile Pro Gly Ser Phe Glu Ala Phe Ala Tyr Ala Gly Asp Leu Asp Lys  
 245 250 255  
 Phe Tyr Gly Thr Gly Tyr Gly Gln Ala Leu Gly Pro Val Gln Gly Val  
 260 265 270  
 Gly Tyr Ile Asn Glu Leu Leu Ala Arg Leu Thr Asn Ser Ala Val Asn  
 275 280 285  
 Asp Asn Thr Gln Thr Asn Arg Thr Leu Asp Ala Ala Pro Asp Thr Phe  
 290 295 300  
 Pro Leu Asn Lys Thr Met Tyr Ala Asp Phe Ser His Asp Asn Leu Met  
 305 310 315 320  
 Val Ala Val Phe Ser Ala Met Gly Leu Phe Arg Gln Ser Ala Pro Leu  
 325 330 335  
 Ser Thr Ser Thr Pro Asp Pro Asn Arg Thr Trp Leu Thr Ser Ser Val  
 340 345 350  
 Val Pro Phe Ser Ala Arg Met Ala Val Glu Arg Leu Ser Cys Ala Gly  
 355 360 365  
 Thr Thr Lys Val Arg Val Leu Val Gln Asp Gln Val Gln Pro Leu Glu  
 370 375 380  
 Phe Cys Gly Gly Asp Gln Asp Gly Leu Cys Ala Leu Asp Lys Phe Val  
 385 390 395 400  
 Glu Ser Gln Ala Tyr Ala Arg Ser Gly Gly Ala Gly Asp Phe Glu Lys  
 405 410 415

Cys Leu Ala Thr Thr Val  
420

<210> 19

<211> 420

<212> PRT

<213> Trametes pubescens

<400> 19

His 1	Ile	Pro	Leu	Arg 5	Asp	Thr	Ser	Ala	Cys 10	Leu	Asp	Val	Thr	Arg 15	Asp
Val	Gln	Gln	Ser 20	Trp	Ser	Met	Tyr	Ser 25	Pro	Tyr	Phe	Pro	Ala 30	Ala	Thr
Tyr	Val 35	Ala	Pro	Pro	Ala	Ser	Cys 40	Gln	Ile	Asn	Gln	Val 45	His	Ile	Ile
Gln	Arg 50	His	Gly	Ala	Arg	Phe 55	Pro	Thr	Ser	Gly	Ala 60	Ala	Lys	Arg	Ile
Gln 65	Thr	Ala	Val	Ala	Lys 70	Leu	Lys	Ala	Ala	Ser 75	Asn	Tyr	Thr	Asp	Pro 80
Leu	Leu	Ala	Phe	Val 85	Thr	Asn	Tyr	Thr	Tyr 90	Ser	Leu	Gly	Gln	Asp 95	Ser
Leu	Val	Glu	Leu 100	Gly	Ala	Thr	Gln	Ser 105	Ser	Glu	Ala	Gly	Gln 110	Glu	Ala
Phe	Thr 115	Arg	Tyr	Ser	Ser	Leu	Val 120	Ser	Ala	Asp	Glu	Leu 125	Pro	Phe	Val
Arg 130	Ala	Ser	Gly	Ser	Asp	Arg 135	Val	Val	Ala	Thr	Ala 140	Asn	Asn	Trp	Thr
Ala 145	Gly	Phe	Ala	Leu	Ala 150	Ser	Ser	Asn	Ser	Ile 155	Thr	Pro	Val	Leu	Ser 160
Val	Ile	Ile	Ser 165	Glu	Ala	Gly	Asn	Asp 170	Thr	Leu	Asp	Asp	Asn 175	Met	Cys
Pro	Ala	Ala	Gly 180	Asp	Ser	Asp	Pro	Gln 185	Val	Asn	Gln	Trp	Leu 190	Ala	Gln
Phe	Ala 195	Pro	Pro	Met	Thr	Ala	Arg 200	Leu	Asn	Ala	Gly	Ala 205	Pro	Gly	Ala
Asn 210	Leu	Thr	Asp	Thr	Asp	Thr 215	Tyr	Asn	Leu	Leu	Thr 220	Leu	Cys	Pro	Phe

## 34

Glu Thr Val Ala Thr Glu Arg Arg Ser Glu Phe Cys Asp Ile Tyr Glu  
 225 230 235 240  
 Glu Leu Gln Ala Glu Asp Ala Phe Ala Tyr Asn Ala Asp Leu Asp Lys  
 245 250 255  
 Phe Tyr Gly Thr Gly Tyr Gly Gln Pro Leu Gly Pro Val Gln Gly Val  
 260 265 270  
 Gly Tyr Ile Asn Glu Leu Ile Ala Arg Leu Thr Ala Gln Asn Val Ser  
 275 280 285  
 Asp His Thr Gln Thr Asn Ser Thr Leu Asp Ser Ser Pro Glu Thr Phe  
 290 295 300  
 Pro Leu Asn Arg Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Gln Met  
 305 310 315 320  
 Val Ala Ile Phe Ser Ala Met Gly Leu Phe Asn Gln Ser Ala Pro Leu  
 325 330 335  
 Asp Pro Thr Thr Pro Asp Pro Ala Arg Thr Phe Leu Val Lys Lys Ile  
 340 345 350  
 Val Pro Phe Ser Ala Arg Met Val Val Glu Arg Leu Asp Cys Gly Gly  
 355 360 365  
 Ala Gln Ser Val Arg Leu Leu Val Asn Asp Ala Val Gln Pro Leu Ala  
 370 375 380  
 Phe Cys Gly Ala Asp Thr Ser Gly Val Cys Thr Leu Asp Ala Phe Val  
 385 390 395 400  
 Glu Ser Gln Ala Tyr Ala Arg Asn Asp Gly Glu Gly Asp Phe Glu Lys  
 405 410 415  
 Cys Phe Ala Thr  
 420

&lt;210&gt; 20

&lt;211&gt; 435

&lt;212&gt; PRT

&lt;213&gt; Agrocybe pediades

&lt;400&gt; 20

Gly Gly Val Val Gln Ala Thr Phe Val Gln Pro Phe Phe Pro Pro Gln  
 1 5 10 15  
 Ile Gln Asp Ser Trp Ala Ala Tyr Thr Pro Tyr Tyr Pro Val Gln Ala  
 20 25 30  
 Tyr Thr Pro Pro Pro Lys Asp Cys Lys Ile Thr Gln Val Asn Ile Ile  
 35 40 45

35

Gln Arg His Gly Ala Arg Phe Pro Thr Ser Gly Ala Gly Thr Arg Ile  
 50 55 60  
 Gln Ala Ala Val Lys Lys Leu Gln Ser Ala Lys Thr Tyr Thr Asp Pro  
 65 70 75 80  
 Arg Leu Asp Phe Leu Thr Asn Tyr Thr Tyr Thr Leu Gly His Asp Asp  
 85 90 95  
 Leu Val Pro Phe Gly Ala Leu Gln Ser Ser Gln Ala Gly Glu Glu Thr  
 100 105 110  
 Phe Gln Arg Tyr Ser Phe Leu Val Ser Lys Glu Asn Leu Pro Phe Val  
 115 120 125  
 Arg Ala Ser Ser Ser Asn Arg Val Val Asp Ser Ala Thr Asn Trp Thr  
 130 135 140  
 Glu Gly Phe Ser Ala Ala Ser His His Val Leu Asn Pro Ile Leu Phe  
 145 150 155 160  
 Val Ile Leu Ser Glu Ser Leu Asn Asp Thr Leu Asp Asp Ala Met Cys  
 165 170 175  
 Pro Asn Ala Gly Ser Ser Asp Pro Gln Thr Gly Ile Trp Thr Ser Ile  
 180 185 190  
 Tyr Gly Thr Pro Ile Ala Asn Arg Leu Asn Gln Gln Ala Pro Gly Ala  
 195 200 205  
 Asn Ile Thr Ala Ala Asp Val Ser Asn Leu Ile Pro Leu Cys Ala Phe  
 210 215 220  
 Glu Thr Ile Val Lys Glu Thr Pro Ser Pro Phe Cys Asn Leu Phe Thr  
 225 230 235 240  
 Pro Glu Glu Phe Ala Gln Phe Glu Tyr Phe Gly Asp Leu Asp Lys Phe  
 245 250 255  
 Tyr Gly Thr Gly Tyr Gly Gln Pro Leu Gly Pro Val Gln Gly Val Gly  
 260 265 270  
 Tyr Ile Asn Glu Leu Leu Ala Arg Leu Thr Glu Met Pro Val Arg Asp  
 275 280 285  
 Asn Thr Gln Thr Asn Arg Thr Leu Asp Ser Ser Pro Leu Thr Phe Pro  
 290 295 300  
 Leu Asp Arg Ser Ile Tyr Ala Asp Leu Ser His Asp Asn Gln Met Ile  
 305 310 315 320  
 Ala Ile Phe Ser Ala Met Gly Leu Phe Asn Gln Ser Ser Pro Leu Asp  
 325 330 335

36

Pro Ser Phe Pro Asn Pro Lys Arg Thr Trp Val Thr Ser Arg Leu Thr  
                   340                  345                  350  
 Pro Phe Ser Ala Arg Met Val Thr Glu Arg Leu Leu Cys Gln Arg Asp  
                   355                  360                  365  
 Gly Thr Gly Ser Gly Gly Pro Ser Arg Ile Met Arg Asn Gly Asn Val  
                   370                  375                  380  
 Gln Thr Phe Val Arg Ile Leu Val Asn Asp Ala Leu Gln Pro Leu Lys  
 385                  390                  395                  400  
 Phe Cys Gly Gly Asp Met Asp Ser Leu Cys Thr Leu Glu Ala Phe Val  
                   405                  410                  415  
 Glu Ser Gln Lys Tyr Ala Arg Glu Asp Gly Gln Gly Asp Phe Glu Lys  
                   420                  425                  430  
 Cys Phe Asp  
                   435

<210> 21  
 <211> 419  
 <212> PRT  
 <213> Peniophora lycii

<400> 21  
 Ser Thr Gln Phe Ser Phe Val Ala Ala Gln Leu Pro Ile Pro Ala Gln  
   1                  5                  10                  15  
 Asn Thr Ser Asn Trp Gly Pro Tyr Asp Pro Phe Phe Pro Val Glu Pro  
                   20                  25                  30  
 Tyr Ala Ala Pro Pro Glu Gly Cys Thr Val Thr Gln Val Asn Leu Ile  
                   35                  40                  45  
 Gln Arg His Gly Ala Arg Trp Pro Thr Ser Gly Ala Arg Ser Arg Gln  
                   50                  55                  60  
 Val Ala Ala Val Ala Lys Ile Gln Met Ala Arg Pro Phe Thr Asp Pro  
 65                  70                  75                  80  
 Lys Tyr Glu Phe Leu Asn Asp Phe Val Tyr Lys Phe Gly Val Ala Asp  
                   85                  90                  95  
 Leu Leu Pro Phe Gly Ala Asn Gln Ser His Gln Thr Gly Thr Asp Met  
                   100                  105                  110  
 Tyr Thr Arg Tyr Ser Thr Leu Phe Glu Gly Gly Asp Val Pro Phe Val  
                   115                  120                  125

37

Arg	Ala	Ala	Gly	Asp	Gln	Arg	Val	Val	Asp	Ser	Ser	Thr	Asn	Trp	Thr	130	135	140	
Ala	Gly	Phe	Gly	Asp	Ala	Ser	Gly	Glu	Thr	Val	Leu	Pro	Thr	Leu	Gln	145	150	155	160
Val	Val	Leu	Gln	Glu	Glu	Gly	Asn	Cys	Thr	Leu	Cys	Asn	Asn	Met	Cys	165	170	175	
Pro	Asn	Glu	Val	Asp	Gly	Asp	Glu	Ser	Thr	Thr	Trp	Leu	Gly	Val	Phe	180	185	190	
Ala	Pro	Asn	Ile	Thr	Ala	Arg	Leu	Asn	Ala	Ala	Ala	Pro	Ser	Ala	Asn	195	200	205	
Leu	Ser	Asp	Ser	Asp	Ala	Leu	Thr	Leu	Met	Asp	Met	Cys	Pro	Phe	Asp	210	215	220	
Thr	Leu	Ser	Ser	Gly	Asn	Ala	Ser	Pro	Phe	Cys	Asp	Leu	Phe	Thr	Ala	225	230	235	240
Glu	Glu	Tyr	Val	Ser	Tyr	Glu	Tyr	Tyr	Tyr	Asp	Leu	Asp	Lys	Tyr	Tyr	245	250	255	
Gly	Thr	Gly	Pro	Gly	Asn	Ala	Leu	Gly	Pro	Val	Gln	Gly	Val	Gly	Tyr	260	265	270	
Val	Asn	Glu	Leu	Leu	Ala	Arg	Leu	Thr	Gly	Gln	Ala	Val	Arg	Asp	Glu	275	280	285	
Thr	Gln	Thr	Asn	Arg	Thr	Leu	Asp	Ser	Asp	Pro	Ala	Thr	Phe	Pro	Leu	290	295	300	
Asn	Arg	Thr	Phe	Tyr	Ala	Asp	Phe	Ser	His	Asp	Asn	Thr	Met	Val	Pro	305	310	315	320
Ile	Phe	Ala	Ala	Leu	Gly	Leu	Phe	Asn	Ala	Thr	Ala	Leu	Asp	Pro	Leu	325	330	335	
Lys	Pro	Asp	Glu	Asn	Arg	Leu	Trp	Val	Asp	Ser	Lys	Leu	Val	Pro	Phe	340	345	350	
Ser	Gly	His	Met	Thr	Val	Glu	Lys	Leu	Ala	Cys	Ser	Gly	Lys	Glu	Ala	355	360	365	
Val	Arg	Val	Leu	Val	Asn	Asp	Ala	Val	Gln	Pro	Leu	Glu	Phe	Cys	Gly	370	375	380	
Gly	Val	Asp	Gly	Val	Cys	Glu	Leu	Ser	Ala	Phe	Val	Glu	Ser	Gln	Thr	385	390	395	400
Tyr	Ala	Arg	Glu	Asn	Gly	Gln	Gly	Asp	Phe	Ala	Lys	Cys	Gly	Phe	Val	405	410	415	

Pro Ser Glu

&lt;210&gt; 22

&lt;211&gt; 369

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence:basidio  
consensus

&lt;400&gt; 22

Ser	Pro	Arg	Thr	Ala	Ala	Gln	Leu	Pro	Ile	Pro	Gln	Gln	Trp	Ser	Pro
1				5					10					15	

Tyr	Ser	Pro	Tyr	Phe	Pro	Val	Ala	Tyr	Ala	Pro	Pro	Ala	Gly	Cys	Gln
			20					25					30		

Ile	Gln	Val	Asn	Ile	Ile	Gln	Arg	His	Gly	Ala	Arg	Phe	Pro	Thr	Ser
		35					40					45			

Gly	Ala	Ala	Thr	Arg	Ile	Gln	Ala	Ala	Val	Ala	Lys	Leu	Gln	Ser	Ala
	50					55					60				

Thr	Asp	Pro	Lys	Leu	Asp	Phe	Leu	Asn	Thr	Tyr	Leu	Gly	Asp	Asp	Leu
65					70					75					80

Val	Pro	Phe	Gly	Ala	Gln	Ser	Ser	Gln	Ala	Gly	Gln	Glu	Ala	Phe	Thr
			85						90					95	

Arg	Tyr	Ser	Leu	Val	Ser	Asp	Asn	Leu	Pro	Phe	Val	Arg	Ala	Ser	Gly
			100					105					110		

Ser	Asp	Arg	Val	Val	Asp	Ser	Ala	Thr	Asn	Trp	Thr	Ala	Gly	Phe	Ala
		115					120					125			

Ala	Ser	Asn	Thr	Pro	Leu	Val	Ile	Leu	Ser	Glu	Gly	Asn	Asp	Thr	Leu
		130				135					140				

Asp	Asp	Asn	Met	Cys	Pro	Ala	Gly	Asp	Ser	Asp	Pro	Gln	Asn	Trp	Leu
145					150					155					160

Ala	Val	Phe	Ala	Pro	Pro	Ile	Thr	Ala	Arg	Leu	Asn	Ala	Ala	Ala	Pro
			165						170					175	

Gly	Ala	Asn	Leu	Thr	Asp	Asp	Ala	Asn	Leu	Leu	Cys	Pro	Phe	Glu	Thr
			180					185					190		

Val	Ser	Glu	Ser	Phe	Cys	Asp	Leu	Phe	Glu	Pro	Glu	Glu	Phe	Ala	Phe
		195					200					205			

39

Tyr Gly Asp Leu Asp Lys Phe Tyr Gly Thr Gly Tyr Gly Gln Pro Leu  
 210 215 220  
 Gly Pro Val Gln Gly Val Gly Tyr Ile Asn Glu Leu Leu Ala Arg Leu  
 225 230 235 240  
 Thr Gln Ala Val Arg Asp Asn Thr Gln Thr Asn Arg Thr Leu Asp Ser  
 245 250 255  
 Ser Pro Thr Phe Pro Leu Asn Arg Thr Phe Tyr Ala Asp Phe Ser His  
 260 265 270  
 Asp Asn Gln Met Val Ala Ile Phe Ser Ala Met Gly Leu Phe Asn Gln  
 275 280 285  
 Ser Ala Pro Leu Asp Pro Ser Pro Asp Pro Asn Arg Thr Trp Val Thr  
 290 295 300  
 Ser Lys Leu Val Pro Phe Ser Ala Arg Met Val Val Glu Arg Leu Cys  
 305 310 315 320  
 Gly Thr Val Arg Val Leu Val Asn Asp Ala Val Gln Pro Leu Glu Phe  
 325 330 335  
 Cys Gly Gly Asp Asp Gly Cys Thr Leu Asp Ala Phe Val Glu Ser Gln  
 340 345 350  
 Tyr Ala Arg Glu Asp Gly Gln Gly Asp Phe Glu Lys Cys Phe Ala Thr  
 355 360 365

Pro

<210> 23  
 <211> 440  
 <212> PRT  
 <213> Thermomyces lanuginosus

&lt;400&gt; 23

Asn Val Asp Ile Ala Arg His Trp Gly Gln Tyr Ser Pro Phe Phe Ser  
 1 5 10 15  
 Leu Ala Glu Val Ser Glu Ile Ser Pro Ala Val Pro Lys Gly Cys Arg  
 20 25 30  
 Val Glu Phe Val Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr  
 35 40 45  
 Ala His Lys Ser Glu Val Tyr Ala Glu Leu Leu Gln Arg Ile Gln Asp  
 50 55 60  
 Thr Ala Thr Glu Phe Lys Gly Asp Phe Ala Phe Leu Arg Asp Tyr Ala  
 65 70 75 80



41

Ala Arg Ala Tyr Val Glu Leu Leu Arg Cys Glu Thr Glu Thr Ser Ser  
 370 375 380

Glu Glu Glu Glu Glu Gly Glu Asp Glu Pro Phe Val Arg Val Leu Val  
 385 390 395 400

Asn Asp Arg Val Val Pro Leu His Gly Cys Arg Val Asp Arg Trp Gly  
 405 410 415

Arg Cys Arg Arg Asp Glu Trp Ile Lys Gly Leu Thr Phe Ala Arg Gln  
 420 425 430

Gly Gly His Trp Asp Arg Cys Phe  
 435 440

<210> 24  
 <211> 441  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: consensus 10

<400> 24

Asn Ser His Ser Cys Asp Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro  
 1 5 10 15

Glu Ile Ser His Leu Trp Gly Gln Tyr Ser Pro Phe Phe Ser Leu Ala  
 20 25 30

Asp Glu Ser Ala Ile Ser Pro Asp Val Pro Lys Gly Cys Arg Val Thr  
 35 40 45

Phe Val Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser  
 50 55 60

Lys Ser Lys Lys Tyr Ser Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala  
 65 70 75 80

Thr Ala Phe Lys Gly Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr  
 85 90 95

Leu Gly Ala Asp Asp Leu Thr Pro Phe Gly Glu Gln Gln Met Val Asn  
 100 105 110

Ser Gly Ile Lys Phe Tyr Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile  
 115 120 125

Val Pro Phe Val Arg Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Ala  
 130 135 140

## 42

Glu Lys Phe Ile Glu Gly Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly  
 145 150 155 160  
 Ala Asn Pro His Gln Ala Ser Pro Val Ile Asn Val Ile Ile Pro Glu  
 165 170 175  
 Gly Ala Gly Tyr Asn Asn Thr Leu Asp His Gly Leu Cys Thr Ala Phe  
 180 185 190  
 Glu Glu Ser Glu Leu Gly Asp Asp Val Glu Ala Asn Phe Thr Ala Val  
 195 200 205  
 Phe Ala Pro Pro Ile Arg Ala Arg Leu Glu Ala His Leu Pro Gly Val  
 210 215 220  
 Asn Leu Thr Asp Glu Asp Val Val Asn Leu Met Asp Met Cys Pro Phe  
 225 230 235 240  
 Asp Thr Val Ala Arg Thr Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys  
 245 250 255  
 Asp Leu Phe Thr His Asp Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser  
 260 265 270  
 Leu Gly Lys Tyr Tyr Gly Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala  
 275 280 285  
 Gln Gly Val Gly Phe Val Asn Glu Leu Ile Ala Arg Leu Thr His Ser  
 290 295 300  
 Pro Val Gln Asp His Thr Ser Thr Asn His Thr Leu Asp Ser Asn Pro  
 305 310 315 320  
 Ala Thr Phe Pro Leu Asn Ala Thr Leu Tyr Ala Asp Phe Ser His Asp  
 325 330 335  
 Asn Thr Met Val Ser Ile Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr  
 340 345 350  
 Lys Pro Leu Ser Thr Thr Ser Val Glu Ser Ile Glu Glu Thr Asp Gly  
 355 360 365  
 Tyr Ala Ala Ser Trp Thr Val Pro Phe Ala Ala Arg Ala Tyr Val Glu  
 370 375 380  
 Met Met Gln Cys Glu Ala Glu Lys Glu Pro Leu Val Arg Val Leu Val  
 385 390 395 400  
 Asn Asp Arg Val Val Pro Leu His Gly Cys Gly Val Asp Lys Leu Gly  
 405 410 415  
 Arg Cys Lys Arg Asp Asp Phe Val Glu Gly Leu Ser Phe Ala Arg Ser  
 420 425 430

43

Gly Gly Asn Trp Glu Glu Cys Phe Ala  
 435 440

<210> 25  
 <211> 1426  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: consensus  
 phytase 10

<220>  
 <221> CDS  
 <222> (12)..(1412)

<220>  
 <221> mat\_peptide  
 <222> (90)..(1412)

<220>  
 <221> sig\_peptide  
 <222> (12)..(89)

<400> 25  
 tatatgaatt c atg ggc gtg ttc gtc gtg cta ctg tcc att gcc acc ttg 50  
 Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu  
 -25 -20 -15

ttc ggt tcc aca tcc ggt acc gcc ttg ggt cct cgt ggt aat tct cac 98  
 Phe Gly Ser Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His  
 -10 -5 -1 1

tct tgt gac act gtt gac ggt ggt tac caa tgt ttc cca gaa att tct 146  
 Ser Cys Asp Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser  
 5 10 15

cac ttg tgg ggt caa tac tct cca ttc ttc tct ttg gct gac gaa tct 194  
 His Leu Trp Gly Gln Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu Ser  
 20 25 30 35

gct att tct cca gac gtt cca aag ggt tgt aga gtt act ttc gtt caa 242  
 Ala Ile Ser Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln  
 40 45 50

gtt ttg tct aga cac ggt gct aga tac cca act tct tct aag tct aag 290  
 Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys  
 55 60 65

aag tac tct gct ttg att gaa gct att caa aag aac gct act gct ttc 338  
 Lys Tyr Ser Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe  
 70 75 80

44

aag ggt aag tac gct ttc ttg aag act tac aac tac act ttg ggt gct	386
Lys Gly Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala	
85 90 95	
gac gac ttg act cca ttc ggt gaa caa caa atg gtt aac tct ggt att	434
Asp Asp Leu Thr Pro Phe Gly Glu Gln Gln Met Val Asn Ser Gly Ile	
100 105 110 115	
aag ttc tac aga aga tac aag gct ttg gct aga aag att gtt cca ttc	482
Lys Phe Tyr Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe	
120 125 130	
gtt aga gct tct ggt tct gac aga gtt att gct tct gct gaa aag ttc	530
Val Arg Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe	
135 140 145	
att gaa ggt ttc caa tct gct aag ttg gct gac cca ggt gct aac cca	578
Ile Glu Gly Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro	
150 155 160	
cac caa gct tct cca gtt att aac gtt att att cca gaa ggt gct ggt	626
His Gln Ala Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly	
165 170 175	
tac aac aac act ttg gac cac ggt ttg tgt act gct ttc gaa gaa tct	674
Tyr Asn Asn Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser	
180 185 190 195	
gaa ttg ggt gac gac gtt gaa gct aac ttc act gct gtt ttc gct cca	722
Glu Leu Gly Asp Asp Val Glu Ala Asn Phe Thr Ala Val Phe Ala Pro	
200 205 210	
cct att aga gct aga ttg gaa gct cac ttg cca ggt gtt aac ttg act	770
Pro Ile Arg Ala Arg Leu Glu Ala His Leu Pro Gly Val Asn Leu Thr	
215 220 225	
gac gaa gac gtt gtt aac ttg atg gac atg tgt cca ttc gac act gtt	818
Asp Glu Asp Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val	
230 235 240	
gct aga act tct gac gct act caa ttg tct cca ttc tgt gac ttg ttc	866
Ala Arg Thr Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe	
245 250 255	
act cac gac gaa tgg att caa tac gac tac ttg caa tct ttg ggt aag	914
Thr His Asp Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys	
260 265 270 275	
tac tac ggt tac ggt gct ggt aac cca ttg ggt cca gct caa ggt gtt	962
Tyr Tyr Gly Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val	
280 285 290	

45

ggt ttc gtt aac gaa ttg att gct aga ttg act cac tct cca gtt caa 1010  
 Gly Phe Val Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln  
                   295                  300                  305

gac cac act tct act aac cac act ttg gac tct aac cca gct act ttc 1058  
 Asp His Thr Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe  
                   310                  315                  320

cca ttg aac gct act ttg tac gct gac ttc tct cac gac aac act atg 1106  
 Pro Leu Asn Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met  
                   325                  330                  335

gtt tct att ttc ttc gct ttg ggt ttg tac aac ggt act aag cca ttg 1154  
 Val Ser Ile Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu  
 340                  345                  350                  355

tct act act tct gtt gaa tct att gaa gaa act gac ggt tac gct gct 1202  
 Ser Thr Thr Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ala Ala  
                   360                  365                  370

tct tgg act gtt cca ttc gct gct aga gct tac gtt gaa atg atg caa 1250  
 Ser Trp Thr Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln  
                   375                  380                  385

tgt gaa gct gaa aag gaa cca ttg gtt aga gtt ttg gtt aac gac aga 1298  
 Cys Glu Ala Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg  
                   390                  395                  400

gtt gtt cca ttg cac ggt tgt ggt gtt gac aag ttg ggt aga tgt aag 1346  
 Val Val Pro Leu His Gly Cys Gly Val Asp Lys Leu Gly Arg Cys Lys  
                   405                  410                  415

aga gac gac ttc gtt gaa ggt ttg tct ttc gct aga tct ggt ggt aac 1394  
 Arg Asp Asp Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn  
 420                  425                  430                  435

tgg gaa gaa tgt ttc gct taagaattca tata 1426  
 Trp Glu Glu Cys Phe Ala  
                   440

&lt;210&gt; 26

&lt;211&gt; 467

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

 <223> Description of Artificial Sequence: consensus  
 phytase 10

&lt;400&gt; 26

 Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser  
           -25                  -20                  -15

 Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp  
 -10                  -5                  -1    1                  5

46

Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp  
 10 15 20  
 Gly Gln Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser  
 25 30 35  
 Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Ser  
 40 45 50  
 Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Lys Tyr Ser  
 55 60 65 70  
 Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys  
 75 80 85  
 Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu  
 90 95 100  
 Thr Pro Phe Gly Glu Gln Gln Met Val Asn Ser Gly Ile Lys Phe Tyr  
 105 110 115  
 Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Val Arg Ala  
 120 125 130  
 Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly  
 135 140 145 150  
 Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro His Gln Ala  
 155 160 165  
 Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly Tyr Asn Asn  
 170 175 180  
 Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser Glu Leu Gly  
 185 190 195  
 Asp Asp Val Glu Ala Asn Phe Thr Ala Val Phe Ala Pro Pro Ile Arg  
 200 205 210  
 Ala Arg Leu Glu Ala His Leu Pro Gly Val Asn Leu Thr Asp Glu Asp  
 215 220 225 230  
 Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr  
 235 240 245  
 Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr His Asp  
 250 255 260  
 Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly  
 265 270 275  
 Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Val  
 280 285 290

47

Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr  
 295 300 305 310  
 Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn  
 315 320 325  
 Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Val Ser Ile  
 330 335 340  
 Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr  
 345 350 355  
 Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ala Ala Ser Trp Thr  
 360 365 370  
 Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Glu Ala  
 375 380 385 390  
 Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
 395 400 405  
 Leu His Gly Cys Gly Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
 410 415 420  
 Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu  
 425 430 435  
 Cys Phe Ala  
 440

<210> 27  
 <211> 437  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: consensus  
 phytase 11

<400> 27  
 Asn Ser His Ser Cys Asp Thr Val Asp Gly Tyr Gln Cys Pro Glu Ile  
 1 5 10 15  
 Ser His Leu Trp Gly Gln Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu  
 20 25 30  
 Ser Ala Ile Ser Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val  
 35 40 45  
 Gln Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser  
 50 55 60

48

Lys Lys Tyr Ser Ala Leu Ile Glu Arg Ile Gln Lys Asn Ala Thr Phe  
 65 70 75 80  
 Lys Gly Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala  
 85 90 95  
 Asp Asp Leu Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile  
 100 105 110  
 Lys Phe Tyr Arg Arg Tyr Lys Ala Leu Ala Arg Asn Ile Val Pro Phe  
 115 120 125  
 Val Arg Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe  
 130 135 140  
 Ile Glu Gly Phe Gln Ser Ala Lys Leu Ala Asp Pro Ala His Gln Ala  
 145 150 155 160  
 Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn  
 165 170 175  
 Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Asp Ser Thr Leu Gly  
 180 185 190  
 Asp Asp Ala Glu Ala Asn Phe Thr Ala Val Phe Ala Pro Pro Ile Arg  
 195 200 205  
 Ala Arg Leu Glu Ala Leu Pro Gly Val Asn Leu Thr Asp Glu Asp Val  
 210 215 220  
 Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr Ser  
 225 230 235 240  
 Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr Ala Asp Glu  
 245 250 255  
 Trp Gln Tyr Asp Tyr Leu Gln Ser Leu Lys Tyr Tyr Gly Tyr Gly Ala  
 260 265 270  
 Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Asn Glu Leu Ile  
 275 280 285  
 Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr Ser Thr Asn His  
 290 295 300  
 Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn Ala Thr Leu Tyr  
 305 310 315 320  
 Ala Asp Phe Ser His Asp Asn Thr Met Val Ser Ile Phe Phe Ala Leu  
 325 330 335  
 Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr Ser Val Glu Ser  
 340 345 350

[illegible]

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<210> 28
<211> 1404
<212> DNA
<213> Artificial Sequence
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<220>
<223> Description of Artificial Sequence: consensus
      phytase 1 thermo 8 q50t, k91a
```

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<220>  
<221> CDS  
<222> (1)..(1401)
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<220>  
<221> mat_peptide  
<222> (79)..(1401)
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<220>  
<221> sig_peptide  
<222> (1)..(78)
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<400> 28																
atg	ggc	gtg	ttc	gtc	gtg	cta	ctg	tcc	att	gcc	acc	ttg	ttc	ggc	tcc	48
Met	Gly	Val	Phe	Val	Val	Leu	Leu	Ser	Ile	Ala	Thr	Leu	Phe	Gly	Ser	
	-25					-20					-15					
aca	tcc	ggc	acc	gcc	ttg	ggc	cct	cgt	ggc	aat	tct	cac	tct	tgt	gac	96
Thr	Ser	Gly	Thr	Ala	Leu	Gly	Pro	Arg	Gly	Asn	Ser	His	Ser	Cys	Asp	
-10					-5				-1	1				5		
act	gtt	gac	ggc	ggc	tac	caa	tgt	ttc	cca	gaa	att	tct	cac	ttg	tgg	144
Thr	Val	Asp	Gly	Gly	Tyr	Gln	Cys	Phe	Pro	Glu	Ile	Ser	His	Leu	Trp	
			10					15					20			

50

ggt acc tac tct cca tac ttc tct ttg gca gac gaa tct gct att tct	192
Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser	
25 30 35	
cca gac gtt cca gac gac tgt aga gtt act ttc gtt caa gtt ttg tct	240
Pro Asp Val Pro Asp Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser	
40 45 50	
aga cac ggt gct aga tac cca act tct tct gcg tct aag gct tac tct	288
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser	
55 60 65 70	
gct ttg att gaa gct att caa aag aac gct act gct ttc aag ggt aag	336
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys	
75 80 85	
tac gct ttc ttg aag act tac aac tac act ttg ggt gct gac gac ttg	384
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu	
90 95 100	
act cca ttc ggt gaa aac caa atg gtt aac tct ggt att aag ttc tac	432
Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile Lys Phe Tyr	
105 110 115	
aga aga tac aag gct ttg gct aga aag att gtt cca ttc att aga gct	480
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala	
120 125 130	
tct ggt tct gac aga gtt att gct tct gct gaa aag ttc att gaa ggt	528
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly	
135 140 145 150	
ttc caa tct gct aag ttg gct gac cca ggt tct caa cca cac caa gct	576
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro His Gln Ala	
155 160 165	
tct cca gtt att aac gtg atc att cca gaa gga tcc ggt tac aac aac	624
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn	
170 175 180	
act ttg gac cac ggt act tgt act gct ttc gaa gac tct gaa tta ggt	672
Thr Leu Asp His Gly Thr Cys Thr Ala Phe Glu Asp Ser Glu Leu Gly	
185 190 195	
gac gac gtt gaa gct aac ttc act gct ttg ttc gct cca gct att aga	720
Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro Ala Ile Arg	
200 205 210	
gct aga ttg gaa gct gac ttg cca ggt gtt act ttg act gac gaa gac	768
Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr Asp Glu Asp	
215 220 225 230	

51

gtt gtt tac ttg atg gac atg tgt cca ttc gac act gtc gct aga act	816
Val Val Tyr Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr	
235 240 245	
tct gac gct act gaa ttg tct cca ttc tgt gct ttg ttc act cac gac	864
Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe Thr His Asp	
250 255 260	
gaa tgg atc caa tac gac tac ttg caa agc ttg ggt aag tac tac ggt	912
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly	
265 270 275	
tac ggt gct ggt aac cca ttg ggt cca gct caa ggt gtt ggt ttc gct	960
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Ala	
280 285 290	
aac gaa ttg att gct aga ttg act cac tct cca gtt caa gac cac act	1008
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr	
295 300 310	
tct act aac cac act ttg gac tct aac cca gct act ttc cca ttg aac	1056
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn	
315 320 325	
gct act ttg tac gct gac ttc tct cac gac aac act atg ata tct att	1104
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Ile Ser Ile	
330 335 340	
ttc ttc gct ttg ggt ttg tac aac ggt acc aag cca ttg tct act act	1152
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr	
345 350 355	
tct gtt gaa tct att gaa gaa act gac ggt tac tct gct tct tgg act	1200
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr	
360 365 370	
gtt cca ttc gct gct aga gct tac gtt gaa atg atg caa tgt caa gct	1248
Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala	
375 380 385 390	
gaa aag gaa cca ttg gtt aga gtt ttg gtt aac gac aga gtt gtt cca	1296
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro	
395 400 405	
ttg cac ggt tgt gct gtt gac aag ttg ggt aga tgt aag aga gac gac	1344
Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp	
410 415 420	
ttc gtt gaa ggt ttg tct ttc gct aga tct ggt ggt aac tgg gct gaa	1392
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu	
425 430 435	

tgt ttc gct taa  
Cys Phe Ala  
440

1404

<210> 29  
<211> 467  
<212> PRT  
<213> Artificial Sequence  
<223> Description of Artificial Sequence: consensus  
phytase 1 thermo 8 q50t, k91a

<400> 29  
Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser  
-25 -20 -15  
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp  
-10 -5 -1 1 5  
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp  
10 15 20  
Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser  
25 30 35  
Pro Asp Val Pro Asp Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser  
40 45 50  
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser  
55 60 65 70  
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys  
75 80 85  
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu  
90 95 100  
Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile Lys Phe Tyr  
105 110 115  
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala  
120 125 130  
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly  
135 140 145 150  
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro His Gln Ala  
155 160 165  
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn  
170 175 180  
Thr Leu Asp His Gly Thr Cys Thr Ala Phe Glu Asp Ser Glu Leu Gly  
185 190 195

Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro Ala Ile Arg  
 200 205 210  
 Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr Asp Glu Asp  
 215 220 225 230  
 Val Val Tyr Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr  
 235 240 245  
 Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe Thr His Asp  
 250 255 260  
 Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly  
 265 270 275  
 Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Ala  
 280 285 290  
 Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr  
 295 300 305 310  
 Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn  
 315 320 325  
 Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Ile Ser Ile  
 330 335 340  
 Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr  
 345 350 355  
 Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr  
 360 365 370  
 Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala  
 375 380 385 390  
 Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
 395 400 405  
 Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
 410 415 420  
 Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu  
 425 430 435  
 Cys Phe Ala  
 440

&lt;210&gt; 30

&lt;211&gt; 1404

&lt;212&gt; DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: consensus  
phytase 10 thermo 3 q50t, k91a

<220>

<221> CDS

<222> (1)..(1401)

<220>

<221> mat\_peptide

<222> (79)..(1401)

<220>

<221> sig\_peptide

<222> (1)..(78)

<400> 30

atg ggc gtg ttc gtc gtg cta ctg tcc att gcc acc ttg ttc ggt tcc	48
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-25 -20 -15	
aca tcc ggt acc gcc ttg ggt cct cgt ggt aac tct cac tct tgt gac	96
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp	
-10 -5 -1 1 5	
act gtt gac ggt ggt tac caa tgt ttc cca gaa att tct cac ttg tgg	144
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp	
10 15 20	
ggt aca tac tct cca ttc ttc tct ttg gct gac gaa tct gct att tct	192
Gly Thr Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser	
25 30 35	
cca gac gtt cca aag ggt tgt aga gtt act ttc gtt caa gtt ttg tct	240
Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Ser	
40 45 50	
aga cac ggt gct aga tac cca act tct tct gcg tct aag gcg tac tct	288
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser	
55 60 65 70	
gct ttg att gaa gct att caa aag aac gct act gct ttc aag ggt aag	336
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys	
75 80 85	
tac gct ttc ttg aag act tac aac tac act ttg ggt gct gac gac ttg	384
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu	
90 95 100	
act cca ttc ggt gaa caa caa atg gtt aac tct ggt att aag ttc tac	432
Thr Pro Phe Gly Glu Gln Gln Met Val Asn Ser Gly Ile Lys Phe Tyr	
105 110 115	

aga aga tac aag gct ttg gct aga aag att gtt cca ttc att aga gct	480
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala	
120 125 130	
tct ggt tct gac aga gtt att gct tct gct gaa aag ttc att gaa ggt	528
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly	
135 140 145 150	
ttc caa tct gct aag ttg gct gac cca ggt gct aac cca cac caa gct	576
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro His Gln Ala	
155 160 165	
tct cca gtt att aac gtt att att cca gaa ggt gct ggt tac aac aac	624
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly Tyr Asn Asn	
170 175 180	
act ttg gac cac ggt ttg tgt act gct ttc gaa gaa tct gaa ttg ggt	672
Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser Glu Leu Gly	
185 190 195	
gac gac gtt gaa gct aac ttc act gct gtt ttc gct cca cca att aga	720
Asp Asp Val Glu Ala Asn Phe Thr Ala Val Phe Ala Pro Pro Ile Arg	
200 205 210	
gct aga ttg gaa gct cac ttg cca ggt gtt aac ttg act gac gaa gac	768
Ala Arg Leu Glu Ala His Leu Pro Gly Val Asn Leu Thr Asp Glu Asp	
215 220 225 230	
gtt gtt aac ttg atg gac atg tgt cca ttc gac act gtt gct aga act	816
Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr	
235 240 245	
tct gac gct act caa ttg tct cca ttc tgt gac ttg ttc act cac gac	864
Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr His Asp	
250 255 260	
gaa tgg att caa tac gac tac ttg caa tct ttg ggt aag tac tac ggt	912
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly	
265 270 275	
tac ggt gct ggt aac cca ttg ggt cca gct caa ggt gtt ggt ttc gtt	960
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Val	
280 285 290	
aac gaa ttg att gct aga ttg act cac tct cca gtt caa gac cac act	1008
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr	
295 300 305 310	
tct act aac cac act ttg gac tct aac cca gct act ttc cca ttg aac	1056
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn	
315 320 325	

56

[illegible]

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<210> 31
<211> 467
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: consensus
      phytase 10 thermo 3 q50t, k91a
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<400> 31
Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser
  -25                      -20                      -15

Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp
-10                      -5                      -1      1                      5

Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp
      10                      15                      20

Gly Thr Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser
      25                      30                      35

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57

Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Ser  
 40 45 50  
 Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser  
 55 60 65 70  
 Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys  
 75 80 85  
 Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu  
 90 95 100  
 Thr Pro Phe Gly Glu Gln Gln Met Val Asn Ser Gly Ile Lys Phe Tyr  
 105 110 115  
 Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala  
 120 125 130  
 Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly  
 135 140 145 150  
 Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro His Gln Ala  
 155 160 165  
 Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly Tyr Asn Asn  
 170 175 180  
 Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser Glu Leu Gly  
 185 190 195  
 Asp Asp Val Glu Ala Asn Phe Thr Ala Val Phe Ala Pro Pro Ile Arg  
 200 205 210  
 Ala Arg Leu Glu Ala His Leu Pro Gly Val Asn Leu Thr Asp Glu Asp  
 215 220 225 230  
 Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr  
 235 240 245  
 Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr His Asp  
 250 255 260  
 Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly  
 265 270 275  
 Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Val  
 280 285 290  
 Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr  
 295 300 305 310  
 Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn  
 315 320 325

58

Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Val Ser Ile  
                   330                  335                  340

Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr  
                   345                  350                  355

Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr  
                   360                  365                  370

Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Glu Ala  
                   375                  380                  385                  390

Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
                   395                  400                  405

Leu His Gly Cys Gly Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
                   410                  415                  420

Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu  
                   425                  430                  435

Cys Phe Ala  
                   440

<210> 32  
 <211> 1404  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Aspergillus  
           fumigatus alpha-mutant

<220>  
 <221> CDS  
 <222> (1)..(1401)

<220>  
 <221> mat\_peptide  
 <222> (79)..(1401)

<220>  
 <221> sig\_peptide  
 <222> (1)..(78)

<400> 32  
 atg ggg gtt ttc gtc gtt cta tta tct atc gcg act ctg ttc ggc agc 48  
 Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser  
           -25                  -20                  -15

59

aca tcg ggc act gcg ctg ggc ccc cgt gga aat cac tcc aag tcc tgc	96
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn His Ser Lys Ser Cys	
-10 -5 -1 1 5	
gat acg gta gac cta ggg tac cag tgc tcc cct gcg act tct cat cta	144
Asp Thr Val Asp Leu Gly Tyr Gln Cys Ser Pro Ala Thr Ser His Leu	
10 15 20	
tgg ggc acg tac tcg cca tac ttt tcg ctc gag gac gag ctg tcc gtg	192
Trp Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Glu Asp Glu Leu Ser Val	
25 30 35	
tcg agt aag ctt ccc aag gat tgc cgg atc acc ttg gta cag gtg cta	240
Ser Ser Lys Leu Pro Lys Asp Cys Arg Ile Thr Leu Val Gln Val Leu	
40 45 50	
tcg cgc cat gga gcg cgg tac cca acc agc tcc aag agc aaa aag tat	288
Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Lys Tyr	
55 60 65 70	
aag aag ctt att acg gcg atc cag gcc aat gcc acc gac ttc aag ggc	336
Lys Lys Leu Ile Thr Ala Ile Gln Ala Asn Ala Thr Asp Phe Lys Gly	
75 80 85	
aag tac gcc ttt ttg aag acg tac aac tat act ctg ggt gcg gat gac	384
Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp	
90 95 100	
ctc act ccc ttt ggg gag cag cag ctg gtg aac tcg ggc atc aag ttc	432
Leu Thr Pro Phe Gly Glu Gln Gln Leu Val Asn Ser Gly Ile Lys Phe	
105 110 115	
tac cag agg tac aag gct ctg gcg cgc agt gtg gtg ccg ttt att cgc	480
Tyr Gln Arg Tyr Lys Ala Leu Ala Arg Ser Val Val Pro Phe Ile Arg	
120 125 130	
gcc tca ggc tcg gac cgg gtt att gct tcg gga gag aag ttc atc gag	528
Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Gly Glu Lys Phe Ile Glu	
135 140 145 150	
ggg ttc cag cag gcg aag ctg gct gat cct ggc gcg acg aac cgc gcc	576
Gly Phe Gln Gln Ala Lys Leu Ala Asp Pro Gly Ala Thr Asn Arg Ala	
155 160 165	
gct ccg gcg att agt gtg att att ccg gag agc gag acg ttc aac aat	624
Ala Pro Ala Ile Ser Val Ile Ile Pro Glu Ser Glu Thr Phe Asn Asn	
170 175 180	
acg ctg gac cac ggt gtg tgc acg aag ttt gag gcg agt cag ctg gga	672
Thr Leu Asp His Gly Val Cys Thr Lys Phe Glu Ala Ser Gln Leu Gly	
185 190 195	

60

gat gag gtt gcg gcc aat ttc act gcg ctc ttt gca ccc gac atc cga	720
Asp Glu Val Ala Ala Asn Phe Thr Ala Leu Phe Ala Pro Asp Ile Arg	
200 205 210	
gct cgc ctc gag aag cat ctt cct ggc gtg acg ctg aca gac gag gac	768
Ala Arg Leu Glu Lys His Leu Pro Gly Val Thr Leu Thr Asp Glu Asp	
215 220 225 230	
gtt gtc agt cta atg gac atg tgt ccg ttt gat acg gta gcg cgc acc	816
Val Val Ser Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr	
235 240 245	
agc gac gca agt cag ctg tca ccg ttc tgt caa ctc ttc act cac aat	864
Ser Asp Ala Ser Gln Leu Ser Pro Phe Cys Gln Leu Phe Thr His Asn	
250 255 260	
gag tgg aag aag tac gac tac ctt cag tcc ttg ggc aag tac tac ggc	912
Glu Trp Lys Lys Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly	
265 270 275	
tac ggc gca ggc aac cct ctg gga ccg gct cag ggg ata ggg ttc acc	960
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Ile Gly Phe Thr	
280 285 290	
aac gag ctg att gcc cgg ttg acg cgt tcg cca gtg cag gac cac acc	1008
Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro Val Gln Asp His Thr	
295 300 305 310	
agc act aac tcg act cta gtc tcc aac ccg gcc acc ttc ccg ttg aac	1056
Ser Thr Asn Ser Thr Leu Val Ser Asn Pro Ala Thr Phe Pro Leu Asn	
315 320 325	
gct acc atg tac gtc gac ttt tca cac gac aac agc atg gtt tcc atc	1104
Ala Thr Met Tyr Val Asp Phe Ser His Asp Asn Ser Met Val Ser Ile	
330 335 340	
ttc ttt gca ttg ggc ctg tac aac ggc act gaa ccc ttg tcc cgg acc	1152
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Glu Pro Leu Ser Arg Thr	
345 350 355	
tcg gtg gaa agc gcc aag gaa ttg gat ggg tat tct gca tcc tgg gtg	1200
Ser Val Glu Ser Ala Lys Glu Leu Asp Gly Tyr Ser Ala Ser Trp Val	
360 365 370	
gtg cct ttc ggc gcg cga gcc tac ttc gag acg atg caa tgc aag tcg	1248
Val Pro Phe Gly Ala Arg Ala Tyr Phe Glu Thr Met Gln Cys Lys Ser	
375 380 385 390	
gaa aag gag cct ctt gtt cgc gct ttg att aat gac cgg gtt gtg cca	1296
Glu Lys Glu Pro Leu Val Arg Ala Leu Ile Asn Asp Arg Val Val Pro	
395 400 405	

61

ctg cat ggc tgc gat gtg gac aag ctg ggg cga tgc aag ctg aat gac 1344  
Leu His Gly Cys Asp Val Asp Lys Leu Gly Arg Cys Lys Leu Asn Asp  
410 415 420

ttt gtc aag gga ttg agt tgg gcc aga tct ggg ggc aac tgg gga gag 1392  
Phe Val Lys Gly Leu Ser Trp Ala Arg Ser Gly Gly Asn Trp Gly Glu  
425 430 435

tgc ttt agt tga 1404  
Cys Phe Ser  
440

<210> 33

<211> 467

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: *Aspergillus fumigatus* alpha-mutant

<400> 33

Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser  
-25 -20 -15

Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn His Ser Lys Ser Cys  
-10 -5 -1 1 5

Asp Thr Val Asp Leu Gly Tyr Gln Cys Ser Pro Ala Thr Ser His Leu  
10 15 20

Trp Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Glu Asp Glu Leu Ser Val  
25 30 35

Ser Ser Lys Leu Pro Lys Asp Cys Arg Ile Thr Leu Val Gln Val Leu  
40 45 50

Ser Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Lys Tyr  
55 60 65 70

Lys Lys Leu Ile Thr Ala Ile Gln Ala Asn Ala Thr Asp Phe Lys Gly  
75 80 85

Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp  
90 95 100

Leu Thr Pro Phe Gly Glu Gln Gln Leu Val Asn Ser Gly Ile Lys Phe  
105 110 115

Tyr Gln Arg Tyr Lys Ala Leu Ala Arg Ser Val Val Pro Phe Ile Arg  
120 125 130

Ala Ser Gly Ser Asp Arg Val Ile Ala Ser Gly Glu Lys Phe Ile Glu  
135 140 145 150

62

Gly Phe Gln Gln Ala Lys Leu Ala Asp Pro Gly Ala Thr Asn Arg Ala  
 155 160 165  
 Ala Pro Ala Ile Ser Val Ile Ile Pro Glu Ser Glu Thr Phe Asn Asn  
 170 175 180  
 Thr Leu Asp His Gly Val Cys Thr Lys Phe Glu Ala Ser Gln Leu Gly  
 185 190 195  
 Asp Glu Val Ala Ala Asn Phe Thr Ala Leu Phe Ala Pro Asp Ile Arg  
 200 205 210  
 Ala Arg Leu Glu Lys His Leu Pro Gly Val Thr Leu Thr Asp Glu Asp  
 215 220 225 230  
 Val Val Ser Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr  
 235 240 245  
 Ser Asp Ala Ser Gln Leu Ser Pro Phe Cys Gln Leu Phe Thr His Asn  
 250 255 260  
 Glu Trp Lys Lys Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly  
 265 270 275  
 Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Ile Gly Phe Thr  
 280 285 290  
 Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro Val Gln Asp His Thr  
 295 300 305 310  
 Ser Thr Asn Ser Thr Leu Val Ser Asn Pro Ala Thr Phe Pro Leu Asn  
 315 320 325  
 Ala Thr Met Tyr Val Asp Phe Ser His Asp Asn Ser Met Val Ser Ile  
 330 335 340  
 Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Glu Pro Leu Ser Arg Thr  
 345 350 355  
 Ser Val Glu Ser Ala Lys Glu Leu Asp Gly Tyr Ser Ala Ser Trp Val  
 360 365 370  
 Val Pro Phe Gly Ala Arg Ala Tyr Phe Glu Thr Met Gln Cys Lys Ser  
 375 380 385 390  
 Glu Lys Glu Pro Leu Val Arg Ala Leu Ile Asn Asp Arg Val Val Pro  
 395 400 405  
 Leu His Gly Cys Asp Val Asp Lys Leu Gly Arg Cys Lys Leu Asn Asp  
 410 415 420  
 Phe Val Lys Gly Leu Ser Trp Ala Arg Ser Gly Gly Asn Trp Gly Glu  
 425 430 435

Cys Phe Ser  
440

<210> 34  
<211> 1426  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: consensus  
phytase 7

<220>  
<221> CDS  
<222> (12)..(1412)

<220>  
<221> mat\_peptide  
<222> (90)..(1412)

<220>  
<221> sig\_peptide  
<222> (12)..(89)

<400> 34  
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Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu  
-25 -20 -15

ttc ggt tcc aca tcc ggt acc gcc ttg ggt cct cgt ggt aat tct cac 98  
Phe Gly Ser Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His  
-10 -5 -1 1

tct tgt gac act gtt gac ggt ggt tac caa tgt ttc cca gaa att tct 146  
Ser Cys Asp Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser  
5 10 15

cac ttg tgg ggt caa tac tct cca tac ttc tct ttg gaa gac gaa tct 194  
His Leu Trp Gly Gln Tyr Ser Pro Tyr Phe Ser Leu Glu Asp Glu Ser  
20 25 30 35

gct att tct cca gac gtt cca gac gac tgt aga gtt act ttc gtt caa 242  
Ala Ile Ser Pro Asp Val Pro Asp Asp Cys Arg Val Thr Phe Val Gln  
40 45 50

gtt ttg tct aga cac ggt gct aga tac cca act gac tct aag ggt aag 290  
Val Leu Ser Arg His Gly Ala Arg Tyr Pro Thr Asp Ser Lys Gly Lys  
55 60 65

aag tac tct gct ttg att gaa gct att caa aag aac gct act gct ttc 338  
Lys Tyr Ser Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe  
70 75 80

aag ggt aag tac gct ttc ttg aag act tac aac tac act ttg ggt gct	386
Lys Gly Lys Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala	
85 90 95	
gac gac ttg act cca ttc ggt gaa aac caa atg gtt aac tct ggt att	434
Asp Asp Leu Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile	
100 105 110 115	
aag ttc tac aga aga tac aag gct ttg gct aga aag att gtt cca ttc	482
Lys Phe Tyr Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe	
120 125 130	
att aga gct tct ggt tct tct aga gtt att gct tct gct gaa aag ttc	530
Ile Arg Ala Ser Gly Ser Ser Arg Val Ile Ala Ser Ala Glu Lys Phe	
135 140 145	
att gaa ggt ttc caa tct gct aag ttg gct gac cca ggt tct caa cca	578
Ile Glu Gly Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro	
150 155 160	
cac caa gct tct cca gtt att gac gtt att att tct gac gct tct tct	626
His Gln Ala Ser Pro Val Ile Asp Val Ile Ile Ser Asp Ala Ser Ser	
165 170 175	
tac aac aac act ttg gac cca ggt act tgt act gct ttc gaa gac tct	674
Tyr Asn Asn Thr Leu Asp Pro Gly Thr Cys Thr Ala Phe Glu Asp Ser	
180 185 190 195	
gaa ttg gct gac act gtt gaa gct aac ttc act gct ttg ttc gct cca	722
Glu Leu Ala Asp Thr Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro	
200 205 210	
gct att aga gct aga ttg gaa gct gac ttg cca ggt gtt act ttg act	770
Ala Ile Arg Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr	
215 220 225	
gac act gaa gtt act tac ttg atg gac atg tgt tct ttc gaa act gtt	818
Asp Thr Glu Val Thr Tyr Leu Met Asp Met Cys Ser Phe Glu Thr Val	
230 235 240	
gct aga act tct gac gct act gaa ttg tct cca ttc tgt gct ttg ttc	866
Ala Arg Thr Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe	
245 250 255	
act cac gac gaa tgg aga cac tac gac tac ttg caa tct ttg aag aag	914
Thr His Asp Glu Trp Arg His Tyr Asp Tyr Leu Gln Ser Leu Lys Lys	
260 265 270 275	
tac tac ggt cac ggt gct ggt aac cca ttg ggt cca act caa ggt gtt	962
Tyr Tyr Gly His Gly Ala Gly Asn Pro Leu Gly Pro Thr Gln Gly Val	
280 285 290	

65

ggt ttc gct aac gaa ttg att gct aga ttg act aga tct cca gtt caa 1010  
Gly Phe Ala Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro Val Gln  
295 300 305

gac cac act tct act aac cac act ttg gac tct aac cca gct act ttc 1058  
Asp His Thr Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe  
310 315 320

cca ttg aac gct act ttg tac gct gac ttc tct cac gac aac ggt att 1106  
Pro Leu Asn Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Gly Ile  
325 330 335

att tct att ttc ttc gct ttg ggt ttg tac aac ggt act gct cca ttg 1154  
Ile Ser Ile Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Ala Pro Leu  
340 345 350 355

tct	act	act	tct	gtt	gaa	tct	att	gaa	gaa	act	gac	ggg	tac	tct	tct	1202
Ser	Thr	Thr	Ser	Val	Glu	Ser	Ile	Glu	Glu	Thr	Asp	Gly	Tyr	Ser	Ser	
				360				365						370		

gct tgg act gtt cca ttc gct tct aga gct tac gtt gaa atg atg caa 1250  
Ala Trp Thr Val Pro Phe Ala Ser Arg Ala Tyr Val Glu Met Met Gln  
375 380 385

tgt caa gct gaa aag gaa cca ttg gtt aga gtt ttg gtt aac gac aga 1298  
Cys Gln Ala Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg  
390 395 400

gtt gtt cca ttg cac ggt tgt gct gtt gac aag ttg ggt aga tgt aag 1346  
Val Val Pro Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys  
405 410 415

aga gac gac ttc gtt gaa ggt ttg tct ttc gct aga tct ggt ggt aac 1394  
Arg Asp Asp Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn  
420 425 430 435

tgg gct gaa tgt ttc gct taagaattca tata 1426  
Trp Ala Glu Cys Phe Ala  
440

<210> 35

<211> 467

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: consensus  
phytase 7

<400> 35

Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser  
-25 -20 -15

Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp  
-10                    -5                    -1    1                    5

Thr	Val	Asp	Gly	Gly	Tyr	Gln	Cys	Phe	Pro	Glu	Ile	Ser	His	Leu	Trp	
			10					15					20			
Gly	Gln	Tyr	Ser	Pro	Tyr	Phe	Ser	Leu	Glu	Asp	Glu	Ser	Ala	Ile	Ser	
		25					30					35				
Pro	Asp	Val	Pro	Asp	Asp	Cys	Arg	Val	Thr	Phe	Val	Gln	Val	Leu	Ser	
	40					45					50					
Arg	His	Gly	Ala	Arg	Tyr	Pro	Thr	Asp	Ser	Lys	Gly	Lys	Lys	Tyr	Ser	
	55				60					65					70	
Ala	Leu	Ile	Glu	Ala	Ile	Gln	Lys	Asn	Ala	Thr	Ala	Phe	Lys	Gly	Lys	
				75					80					85		
Tyr	Ala	Phe	Leu	Lys	Thr	Tyr	Asn	Tyr	Thr	Leu	Gly	Ala	Asp	Asp	Leu	
			90					95					100			
Thr	Pro	Phe	Gly	Glu	Asn	Gln	Met	Val	Asn	Ser	Gly	Ile	Lys	Phe	Tyr	
		105					110					115				
Arg	Arg	Tyr	Lys	Ala	Leu	Ala	Arg	Lys	Ile	Val	Pro	Phe	Ile	Arg	Ala	
	120					125					130					
Ser	Gly	Ser	Ser	Arg	Val	Ile	Ala	Ser	Ala	Glu	Lys	Phe	Ile	Glu	Gly	
135					140					145					150	
Phe	Gln	Ser	Ala	Lys	Leu	Ala	Asp	Pro	Gly	Ser	Gln	Pro	His	Gln	Ala	
				155					160					165		
Ser	Pro	Val	Ile	Asp	Val	Ile	Ile	Ser	Asp	Ala	Ser	Ser	Tyr	Asn	Asn	
			170					175					180			
Thr	Leu	Asp	Pro	Gly	Thr	Cys	Thr	Ala	Phe	Glu	Asp	Ser	Glu	Leu	Ala	
		185					190					195				
Asp	Thr	Val	Glu	Ala	Asn	Phe	Thr	Ala	Leu	Phe	Ala	Pro	Ala	Ile	Arg	
	200					205					210					
Ala	Arg	Leu	Glu	Ala	Asp	Leu	Pro	Gly	Val	Thr	Leu	Thr	Asp	Thr	Glu	
215					220					225					230	
Val	Thr	Tyr	Leu	Met	Asp	Met	Cys	Ser	Phe	Glu	Thr	Val	Ala	Arg	Thr	
				235					240					245		
Ser	Asp	Ala	Thr	Glu	Leu	Ser	Pro	Phe	Cys	Ala	Leu	Phe	Thr	His	Asp	
			250					255					260			
Glu	Trp	Arg	His	Tyr	Asp	Tyr	Leu	Gln	Ser	Leu	Lys	Lys	Tyr	Tyr	Gly	
		265					270					275				
His	Gly	Ala	Gly	Asn	Pro	Leu	Gly	Pro	Thr	Gln	Gly	Val	Gly	Phe	Ala	
						285					290					

67

Asn Glu Leu Ile Ala Arg Leu Thr Arg Ser Pro Val Gln Asp His Thr  
 295 300 305 310  
 Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn  
 315 320 325  
 Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Gly Ile Ile Ser Ile  
 330 335 340  
 Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Ala Pro Leu Ser Thr Thr  
 345 350 355  
 Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ser Ala Trp Thr  
 360 365 370  
 Val Pro Phe Ala Ser Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala  
 375 380 385 390  
 Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
 395 400 405  
 Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
 410 415 420  
 Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu  
 425 430 435  
 Cys Phe Ala  
 440

<210> 36  
 <211> 467  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: consensus  
 phytase 12

<400> 36  
 Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser  
 1 5 10 15  
 Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp  
 20 25 30  
 Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser Ser Asn Trp  
 35 40 45  
 Ser Pro Tyr Ser Pro Tyr Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser  
 50 55 60

68

Pro	Asp	Val	Pro	Lys	Gly	Cys	Arg	Val	Thr	Phe	Val	Gln	Val	Leu	Gln	65	70	75	80
Arg	His	Gly	Ala	Arg	Phe	Pro	Thr	Ser	Gly	Ala	Ala	Thr	Arg	Ile	Ser	85	90	95	
Ala	Leu	Ile	Glu	Ala	Ile	Gln	Lys	Asn	Ala	Thr	Ala	Phe	Lys	Gly	Lys	100	105	110	
Tyr	Ala	Phe	Leu	Lys	Thr	Tyr	Asn	Tyr	Thr	Leu	Gly	Ala	Asp	Asp	Leu	115	120	125	
Val	Pro	Phe	Gly	Ala	Asn	Gln	Ser	Ser	Gln	Ala	Gly	Ile	Lys	Phe	Tyr	130	135	140	
Arg	Arg	Tyr	Lys	Ala	Leu	Ala	Arg	Lys	Ile	Val	Pro	Phe	Ile	Arg	Ala	145	150	155	160
Ser	Gly	Ser	Asp	Arg	Val	Ile	Asp	Ser	Ala	Thr	Asn	Trp	Ile	Glu	Gly	165	170	175	
Phe	Gln	Ser	Ala	Lys	Leu	Ala	Asp	Pro	Gly	Ala	Asn	Pro	His	Gln	Ala	180	185	190	
Ser	Pro	Val	Ile	Asn	Val	Ile	Ile	Pro	Glu	Gly	Ala	Gly	Tyr	Asn	Asn	195	200	205	
Thr	Leu	Asp	His	Gly	Leu	Cys	Thr	Ala	Phe	Glu	Glu	Ser	Glu	Leu	Gly	210	215	220	
Asp	Asp	Val	Glu	Ala	Asn	Phe	Thr	Ala	Val	Phe	Ala	Pro	Pro	Ile	Arg	225	230	235	240
Ala	Arg	Leu	Glu	Ala	His	Leu	Pro	Gly	Val	Asn	Leu	Thr	Asp	Glu	Asp	245	250	255	
Val	Val	Asn	Leu	Met	Asp	Met	Cys	Pro	Phe	Asp	Thr	Val	Ala	Arg	Thr	260	265	270	
Ser	Asp	Ala	Thr	Glu	Leu	Ser	Pro	Phe	Cys	Asp	Leu	Phe	Thr	His	Asp	275	280	285	
Glu	Trp	Ile	Gln	Tyr	Asp	Tyr	Leu	Gly	Asp	Leu	Asp	Lys	Tyr	Tyr	Gly	290	295	300	
Thr	Gly	Ala	Gly	Asn	Pro	Leu	Gly	Pro	Ala	Gln	Gly	Val	Gly	Phe	Val	305	310	315	320
Asn	Glu	Leu	Ile	Ala	Arg	Leu	Thr	His	Ser	Pro	Val	Gln	Asp	His	Thr	325	330	335	
Ser	Thr	Asn	His	Thr	Leu	Asp	Ser	Asn	Pro	Ala	Thr	Phe	Pro	Leu	Asn	340	345	350	

69

Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Val Ala Ile  
 355 360 365

Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr  
 370 375 380

Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Leu  
 385 390 395 400

Val Pro Phe Ser Ala Arg Met Tyr Val Glu Met Met Gln Cys Glu Ala  
 405 410 415

Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
 420 425 430

Leu His Gly Cys Gly Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
 435 440 445

Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu  
 450 455 460

Cys Phe Ala  
 465

<210> 37  
 <211> 26  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: primer

<400> 37  
 tatatgaatt catgggcgtg ttcgtc

26

<210> 38  
 <211> 22  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: primer

<400> 38  
 tgaaaagtgc attgaagggtt tc

22

<210> 39  
 <211> 22  
 <212> DNA  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 39

tcttcgaaag cagtacacaa ac

22

<210> 40

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 40

tatatgaatt cttaagcgaa ac

22

<210> 41

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 41

cacttggtggg gtacctactc tccatacttc tc

32

<210> 42

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 42

gggtcaatact ctccattctt ctcttttgga g

31

<210> 43

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 43

catacttctc tttggcagac gaatctgc

28

<210> 44  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 44  
ctccagacgt cccaaaggac tgtagagtta c

31

<210> 45  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 45  
ctccagacgt cccagacggc tgtagagtta c

31

<210> 46  
<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 46  
gatacccaac ttcttctgcg tctaaggctt actctg

36

<210> 47  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 47  
cttctaagtc taagaagtac tctgctttg

29

<210> 48  
<211> 41  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 48  
gcttactctg ctttgattga acggattcaa aagaacgcta c 41

<210> 49  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 49  
ccattcggtg aacagcaa at ggttaactc 29

<210> 50  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 50  
gatacaaggc tctcgcgaga aacattgttc 30

<210> 51  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 51  
gattgttcca ttcgtgcgcg cttctggttc 30

<210> 52  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 52  
ctccagttat taacgtgatc attccagaag g 31

<210> 53  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 53  
ggctgaccca ggggcccaac cacaccaagc

30

<210> 54  
<211> 32  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 54  
cactttggac catggtcttt gtactgcttt cg

32

<210> 55  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 55  
gctttcgaag actctaccct aggtgacgac gttg

34

<210> 56  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 56  
ggtgacgacg ctgaagctaa cttcac

26

<210> 57  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 57  
ctaacttcac cgcggtgttc gctccag

27

<210> 58  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 58  
gctttgttcg ctccacctat tagagctaga ttgg

34

<210> 59  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 59  
gccaggtggt aacttgactg acgaag

26

<210> 60  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 60  
gacgaagacg tcgttaactt gatggac

27

<210> 61  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 61  
gtccattcga cactgtcgct agaacttc

28

<210> 62  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 62  
ctgacgctac tcagctgtct ccattc

26

<210> 63  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 63  
gtctccattc tgtgatttgt tcactcac

28

<210> 64  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 64  
gctttgttca ccgcggacga atggag

26

<210> 65  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 65  
cacgacgaat ggatccaata cgactac

27

<210> 66  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 66  
gacgaatgga gagcgtacga ctacttg

27

<210> 67  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 67  
ggtgttggtt tcgttaacga attgattgc

29

<210> 68  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 68  
gctagattga ctcactctcc agttcaag

28

<210> 69  
<211> 32  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 69  
ctcacgacaa cactatgata tctattttct tc

32

<210> 70  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 70  
cgacaactcc atggtttcta ttttcttcgc

30

<210> 71  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 71  
gtacaacggt accaagccat tgtctac

27

<210> 72  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 72  
ctgacgggta cgctgcttct tggac

25

<210> 73  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 73  
ctgttcatt cgctgctaga gcttac

26

<210> 74  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 74  
gatgcaatgt gaagctgaaa aggaacc

27

<210> 75  
<211> 26  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 75  
cacggttggtg gtgtcgacaa gttggg

26

<210> 76  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 76  
gatctggtgg caattgggag gaatgtttcg

30

<210> 77  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 77  
cacgtactcg ccatactttt cgctcgag

28

<210> 78  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 78  
ccatactttt cgctcgcgga cgagctgtcc gtg

33

<210> 79  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 79  
gtataagaag cttattacgg cgatccaggc c

31

<210> 80  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 80  
cttcaagggc aagtacgcct ttttgaagac g

31

<210> 81  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 81  
catccgagct cgcctcgaga agcatcttc

29

<210> 82  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 82  
ctaatggatg tgtccgtttg atacggtag

29

<210> 83  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: primer

<400> 83  
gtggaagaag tacgactacc ttcagtc

27

<210> 84  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 84

gcccggttga cgcattcgcc agtgcagg

28

<210> 85

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 85

cacacgacaa caccatgggt tccatcttc

29

<210> 86

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 86

gtggtgcctt tcgccgcgcg agcctacttc

30

<210> 87

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 87

tatatcatga gcgtgttcgt cgtgctactg ttc

33

<210> 88

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: primer

<400> 88

acccgactta caaagcgaat tctatagata tat

33

<210> 89  
 <211> 33  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: primer

<400> 89  
 acccttctta caaagcgaat tctatagata tat 33

<210> 90  
 <211> 1404  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence:  
 Consensus-phytase-3-thermo-11-Q50T

<220>  
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 <222> (1)..(1401)

<220>  
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 <222> (1)..(69)

<220>  
 <221> mat\_peptide  
 <222> (70)..(1401)

<400> 90  
 atg ggc gtg ttc gtc gtg cta ctg tcc att gcc acc ttg ttc ggt tcc 48  
 Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser  
 -20 -15 -10

aca tcc ggt acc gcc ttg ggt cct cgt ggt aat tct cac tct tgt gac 96  
 Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp  
 -5 -1 1 5

act gtt gac ggt ggt tac caa tgt ttc cca gaa att tct cac ttg tgg 144  
 Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp  
 10 15 20 25

ggt acc tac tct cca tac ttc tct ttg gca gac gaa tct gct att tct 192  
 Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser  
 30 35 40

cca gac gtc cca aag gac tgt aga gtt act ttc gtt caa gtt ttg tct 240  
 Pro Asp Val Pro Lys Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser  
 45 50 55

aga cac ggt gct aga tac cca act tct tct aag tct aag gct tac tct	288
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Ala Tyr Ser	
60 65 70	
gct ttg att gaa gct att caa aag aac gct act gct ttc aag ggt aag	336
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys	
75 80 85	
tac gct ttc ttg aag act tac aac tac act ttg ggt gct gac gac ttg	384
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu	
90 95 100 105	
act cca ttc ggt gaa aac caa atg gtt aac tct ggt att aag ttc tac	432
Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile Lys Phe Tyr	
110 115 120	
aga aga tac aag gct ttg gct aga aag att gtt cca ttc att aga gct	480
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala	
125 130 135	
tct ggt tct gac aga gtt att gct tct gct gaa aag ttc att gaa ggt	528
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly	
140 145 150	
ttc caa tct gct aag ttg gct gac cca ggt tct caa cca cac caa gct	576
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro His Gln Ala	
155 160 165	
tct cca gtt att aac gtg atc att cca gaa gga tcc ggt tac aac aac	624
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn	
170 175 180 185	
act ttg gac cat ggt ctt tgt act gct ttc gaa gac tct acc cta ggt	672
Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Asp Ser Thr Leu Gly	
190 195 200	
gac gac gtt gaa gct aac ttc act gct ttg ttc gct cca gct att aga	720
Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro Ala Ile Arg	
205 210 215	
gct aga ttg gaa gct gac ttg cca ggt gtt act ttg act gac gaa gac	768
Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr Asp Glu Asp	
220 225 230	
gtt gtt tac ttg atg gac atg tgt cca ttc gac act gtc gct aga act	816
Val Val Tyr Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr	
235 240 245	
tct gac gct act gaa ttg tct cca ttc tgt gct ttg ttc act cac gac	864
Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe Thr His Asp	
250 255 260 265	

83

gaa tgg atc caa tac gac tac ttg caa agc ttg ggt aag tac tac ggt	912
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly	
270 275 280	
tac ggt gct ggt aac cca ttg ggt cca gct caa ggt gtt ggt ttc gct	960
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Ala	
285 290 295	
aac gaa ttg att gct aga ttg act cac tct cca gtt caa gac cac act	1008
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr	
300 305 310	
tct act aac cac act ttg gac tct aac cca gct act ttc cca ttg aac	1056
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn	
315 320 325	
gct act ttg tac gct gac ttc tct cac gac aac act atg ata tct att	1104
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Ile Ser Ile	
330 335 340 345	
ttc ttc gct ttg ggt ttg tac aac ggt acc aag cca ttg tct act act	1152
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr	
350 355 360	
tct gtt gaa tct att gaa gaa act gac ggt tac tct gct tct tgg act	1200
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr	
365 370 375	
gtt cca ttc gct gct aga gct tac gtt gaa atg atg caa tgt caa gct	1248
Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala	
380 385 390	
gaa aag gaa cca ttg gtt aga gtt ttg gtt aac gac aga gtt gtt cca	1296
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro	
395 400 405	
ttg cac ggt tgt gct gtt gac aag ttg ggt aga tgt aag aga gac gac	1344
Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp	
410 415 420 425	
ttc gtt gaa ggt ttg tct ttc gct aga tct ggt ggt aac tgg gct gaa	1392
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu	
430 435 440	
tgt ttc gct taa	1404
Cys Phe Ala	

&lt;210&gt; 91

&lt;211&gt; 467

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;223&gt; Description of Artificial Sequence:

Consensus-phytase-3-thermo-11-Q50T

&lt;400&gt; 91

Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser  
                   -20                                  -15                                  -10

Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp  
                   -5                                  -1   1                                  5

Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp  
 10                                  15                                  20                                  25

Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser  
                                   30                                  35                                  40

Pro Asp Val Pro Lys Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser  
                   45                                  50                                  55

Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Ala Tyr Ser  
                   60                                  65                                  70

Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys  
                   75                                  80                                  85

Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu  
 90                                  95                                  100                                  105

Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile Lys Phe Tyr  
                                   110                                  115                                  120

Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala  
                   125                                  130                                  135

Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly  
                   140                                  145                                  150

Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro His Gln Ala  
                   155                                  160                                  165

Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn  
 170                                  175                                  180                                  185

Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Asp Ser Thr Leu Gly  
                                   190                                  195                                  200

Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro Ala Ile Arg  
                   205                                  210                                  215

Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr Asp Glu Asp  
                   220                                  225                                  230

Val Val Tyr Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr  
                   235                                  240                                  245

85

Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe Thr His Asp  
 250 255 260 265  
 Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly  
 270 275 280  
 Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Ala  
 285 290 295  
 Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr  
 300 305 310  
 Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn  
 315 320 325  
 Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Ile Ser Ile  
 330 335 340 345  
 Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr  
 350 355 360  
 Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr  
 365 370 375  
 Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala  
 380 385 390  
 Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
 395 400 405  
 Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
 410 415 420 425  
 Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu  
 430 435 440  
 Cys Phe Ala

&lt;210&gt; 92

&lt;211&gt; 1404

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

 <223> Description of Artificial Sequence: Consensus  
 phytase-3-thermo-11-Q50T-K91A

&lt;220&gt;

&lt;221&gt; sig\_peptide

&lt;222&gt; (1)..(69)

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(1401)

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (70)..(1401)

&lt;400&gt; 92

atg ggc gtg ttc gtc gtg cta ctg tcc att gcc acc ttg ttc ggt tcc	48
Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser	
-20 -15 -10	
aca tcc ggt acc gcc ttg ggt cct cgt ggt aat tct cac tct tgt gac	96
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp	
-5 -1 1 5	
act gtt gac ggt ggt tac caa tgt ttc cca gaa att tct cac ttg tgg	144
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp	
10 15 20 25	
ggt acc tac tct cca tac ttc tct ttg gca gac gaa tct gct att tct	192
Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser	
30 35 40	
cca gac gtc cca aag gac tgt aga gtt act ttc gtt caa gtt ttg tct	240
Pro Asp Val Pro Lys Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser	
45 50 55	
aga cac ggt gct aga tac cca act tct tct gcg tct aag gct tac tct	288
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser	
60 65 70	
gct ttg att gaa gct att caa aag aac gct act gct ttc aag ggt aag	336
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys	
75 80 85	
tac gct ttc ttg aag act tac aac tac act ttg ggt gct gac gac ttg	384
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu	
90 95 100 105	
act cca ttc ggt gaa aac caa atg gtt aac tct ggt att aag ttc tac	432
Thr Pro Phe Gly Glu Asn Gln Met Val Asn Ser Gly Ile Lys Phe Tyr	
110 115 120	
aga aga tac aag gct ttg gct aga aag att gtt cca ttc att aga gct	480
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala	
125 130 135	
tct ggt tct gac aga gtt att gct tct gct gaa aag ttc att gaa ggt	528
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly	
140 145 150	

87

ttc caa tct gct aag ttg gct gac cca ggt tct caa cca cac caa gct	576
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ser Gln Pro His Gln Ala	
155 160 165	
tct cca gtt att aac gtg atc att cca gaa gga tcc ggt tac aac aac	624
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ser Gly Tyr Asn Asn	
170 175 180 185	
act ttg gac cat ggt ctt tgt act gct ttc gaa gac tct acc cta ggt	672
Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Asp Ser Thr Leu Gly	
190 195 200	
gac gac gtt gaa gct aac ttc act gct ttg ttc gct cca gct att aga	720
Asp Asp Val Glu Ala Asn Phe Thr Ala Leu Phe Ala Pro Ala Ile Arg	
205 210 215	
gct aga ttg gaa gct gac ttg cca ggt gtt act ttg act gac gaa gac	768
Ala Arg Leu Glu Ala Asp Leu Pro Gly Val Thr Leu Thr Asp Glu Asp	
220 225 230	
gtt gtt tac ttg atg gac atg tgt cca ttc gac act gtc gct aga act	816
Val Val Tyr Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr	
235 240 245	
tct gac gct act gaa ttg tct cca ttc tgt gct ttg ttc act cac gac	864
Ser Asp Ala Thr Glu Leu Ser Pro Phe Cys Ala Leu Phe Thr His Asp	
250 255 260 265	
gaa tgg atc caa tac gac tac ttg cca agc ttg ggt aag tac tac ggt	912
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly	
270 275 280	
tac ggt gct ggt aac cca ttg ggt cca gct cca ggt gtt ggt ttc gct	960
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Ala	
285 290 295	
aac gaa ttg att gct aga ttg act cac tct cca gtt caa gac cac act	1008
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr	
300 305 310	
tct act aac cac act ttg gac tct aac cca gct act ttc cca ttg aac	1056
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn	
315 320 325	
gct act ttg tac gct gac ttc tct cac gac aac act atg ata tct att	1104
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Ile Ser Ile	
330 335 340 345	
ttc ttc gct ttg ggt ttg tac aac ggt acc aag cca ttg tct act act	1152
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr	
350 355 360	

88

tct gtt gaa tct att gaa gaa act gac ggt tac tct gct tct tgg act	1200
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr	
365 370 375	
gtt cca ttc gct gct aga gct tac gtt gaa atg atg caa tgt caa gct	1248
Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Gln Ala	
380 385 390	
gaa aag gaa cca ttg gtt aga gtt ttg gtt aac gac aga gtt gtt cca	1296
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro	
395 400 405	
ttg cac ggt tgt gct gtt gac aag ttg ggt aga tgt aag aga gac gac	1344
Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp	
410 415 420 425	
ttc gtt gaa ggt ttg tct ttc gct aga tct ggt ggt aac tgg gct gaa	1392
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Ala Glu	
430 435 440	
tgt ttc gct taa	1404
Cys Phe Ala	

&lt;210&gt; 93

&lt;211&gt; 467

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

<223> Description of Artificial Sequence: Consensus  
 phytase-3-thermo-11-Q50T-K91A

&lt;400&gt; 93

Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser	
-20 -15 -10	
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp	
-5 -1 1 5	
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp	
10 15 20 25	
Gly Thr Tyr Ser Pro Tyr Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser	
30 35 40	
Pro Asp Val Pro Lys Asp Cys Arg Val Thr Phe Val Gln Val Leu Ser	
45 50 55	
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser	
60 65 70	
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys	
75 80 85	

89

Tyr	Ala	Phe	Leu	Lys	Thr	Tyr	Asn	Tyr	Thr	Leu	Gly	Ala	Asp	Asp	Leu	90	95	100	105
Thr	Pro	Phe	Gly	Glu	Asn	Gln	Met	Val	Asn	Ser	Gly	Ile	Lys	Phe	Tyr	110	115	120	
Arg	Arg	Tyr	Lys	Ala	Leu	Ala	Arg	Lys	Ile	Val	Pro	Phe	Ile	Arg	Ala	125	130	135	
Ser	Gly	Ser	Asp	Arg	Val	Ile	Ala	Ser	Ala	Glu	Lys	Phe	Ile	Glu	Gly	140	145	150	
Phe	Gln	Ser	Ala	Lys	Leu	Ala	Asp	Pro	Gly	Ser	Gln	Pro	His	Gln	Ala	155	160	165	
Ser	Pro	Val	Ile	Asn	Val	Ile	Ile	Pro	Glu	Gly	Ser	Gly	Tyr	Asn	Asn	170	175	180	185
Thr	Leu	Asp	His	Gly	Leu	Cys	Thr	Ala	Phe	Glu	Asp	Ser	Thr	Leu	Gly	190	195	200	
Asp	Asp	Val	Glu	Ala	Asn	Phe	Thr	Ala	Leu	Phe	Ala	Pro	Ala	Ile	Arg	205	210	215	
Ala	Arg	Leu	Glu	Ala	Asp	Leu	Pro	Gly	Val	Thr	Leu	Thr	Asp	Glu	Asp	220	225	230	
Val	Val	Tyr	Leu	Met	Asp	Met	Cys	Pro	Phe	Asp	Thr	Val	Ala	Arg	Thr	235	240	245	
Ser	Asp	Ala	Thr	Glu	Leu	Ser	Pro	Phe	Cys	Ala	Leu	Phe	Thr	His	Asp	250	255	260	265
Glu	Trp	Ile	Gln	Tyr	Asp	Tyr	Leu	Gln	Ser	Leu	Gly	Lys	Tyr	Tyr	Gly	270	275	280	
Tyr	Gly	Ala	Gly	Asn	Pro	Leu	Gly	Pro	Ala	Gln	Gly	Val	Gly	Phe	Ala	285	290	295	
Asn	Glu	Leu	Ile	Ala	Arg	Leu	Thr	His	Ser	Pro	Val	Gln	Asp	His	Thr	300	305	310	
Ser	Thr	Asn	His	Thr	Leu	Asp	Ser	Asn	Pro	Ala	Thr	Phe	Pro	Leu	Asn	315	320	325	
Ala	Thr	Leu	Tyr	Ala	Asp	Phe	Ser	His	Asp	Asn	Thr	Met	Ile	Ser	Ile	330	335	340	345
Phe	Phe	Ala	Leu	Gly	Leu	Tyr	Asn	Gly	Thr	Lys	Pro	Leu	Ser	Thr	Thr	350	355	360	
Ser	Val	Glu	Ser	Ile	Glu	Glu	Thr	Asp	Gly	Tyr	Ser	Ala	Ser	Trp	Thr	365	370	375	

90

[illegible]

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<210> 94
<211> 1404
<212> DNA
<213> Artificial Sequence
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<220>
<223> Description of Artificial Sequence: Consensus
phytase-10-thermo-5-Q50T
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<220>
<221> sig_peptide
<222> (1)..(69)
```

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<220>  
<221> CDS  
<222> (1)..(1401)
```

```
<220>
<221> mat_peptide
<222> (70)..(1401)
```

<400> 94																
atg	ggc	gtg	ttc	gtc	gtg	cta	ctg	tcc	att	gcc	acc	ttg	ttc	ggg	tcc	48
Met	Gly	Val	Phe	Val	Val	Leu	Leu	Ser	Ile	Ala	Thr	Leu	Phe	Gly	Ser	
			-20					-15					-10			
aca	tcc	ggg	acc	gcc	ttg	ggg	cct	cgt	ggg	aat	tct	cac	tct	tgt	gac	96
Thr	Ser	Gly	Thr	Ala	Leu	Gly	Pro	Arg	Gly	Asn	Ser	His	Ser	Cys	Asp	
		-5				-1	1				5					
act	gtt	gac	ggg	ggg	tac	caa	tgt	ttc	cca	gaa	att	tct	cac	ttg	tgg	144
Thr	Val	Asp	Gly	Gly	Tyr	Gln	Cys	Phe	Pro	Glu	Ile	Ser	His	Leu	Trp	
10					15					20				25		
ggg	aca	tac	tct	cca	ttc	ttc	tct	ttg	gct	gac	gaa	tct	gct	att	tct	192
Gly	Thr	Tyr	Ser	Pro	Phe	Phe	Ser	Leu	Ala	Asp	Glu	Ser	Ala	Ile	Ser	
				30				35						40		

cca gac gtt cca aag ggt tgt aga gtt act ttc gtt caa gtt ttg tct	240
Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Ser	
45 50 55	
aga cac ggt gct aga tac cca act tct tct aag tct aag gct tac tct	288
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Lys Ser Lys Ala Tyr Ser	
60 65 70	
gct ttg att gaa gct att caa aag aac gct act gct ttc aag ggt aag	336
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys	
75 80 85	
tac gct ttc ttg aag act tac aat tac act ttg ggt gct gac gac ttg	384
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu	
90 95 100 105	
act cca ttc ggt gaa caa caa atg gtt aac tct ggt att aag ttc tac	432
Thr Pro Phe Gly Glu Gln Gln Met Val Asn Ser Gly Ile Lys Phe Tyr	
110 115 120	
aga aga tac aag gct ttg gct aga aag att gtt cca ttc att aga gct	480
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala	
125 130 135	
tct ggt tct gac aga gtt att gct tct gcc gaa aag ttc att gaa ggt	528
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly	
140 145 150	
ttc caa tct gct aag ttg gct gac cca ggt gct aac cca cac caa gct	576
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro His Gln Ala	
155 160 165	
tct cca gtt att aac gtt att att cca gaa ggt gct ggt tac aac aac	624
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly Tyr Asn Asn	
170 175 180 185	
act ttg gac cac ggt ttg tgt act gct ttc gaa gaa tct acc cta ggt	672
Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser Thr Leu Gly	
190 195 200	
gac gac gtt gaa gct aac ttc act gct gtt ttc gct cca cca att aga	720
Asp Asp Val Glu Ala Asn Phe Thr Ala Val Phe Ala Pro Pro Ile Arg	
205 210 215	
gct aga ttg gaa gct cac ttg cca ggt gtt aac ttg act gac gaa gac	768
Ala Arg Leu Glu Ala His Leu Pro Gly Val Asn Leu Thr Asp Glu Asp	
220 225 230	
gtt gtt aac ttg atg gac atg tgt cca ttc gac act gtt gct aga act	816
Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr	
235 240 245	

92

tct gac gct act caa ttg tct cca ttc tgt gac ttg ttc act cac gac	864
Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr His Asp	
250 255 260 265	
gaa tgg att caa tac gac tac ttg caa tct ttg ggt aag tac tac ggt	912
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly	
270 275 280	
tac ggt gct ggt aac cca ttg ggt cca gct caa ggt gtt ggt ttc gtt	960
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Val	
285 290 295	
aac gaa ttg att gct aga ttg act cac tct cca gtt caa gac cac act	1008
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr	
300 305 310	
tct act aac cac act ttg gac tct aac cca gct act ttc cca ttg aac	1056
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn	
315 320 325	
gct act ttg tac gct gac ttc tct cac gac aac act atg gtt tct att	1104
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Val Ser Ile	
330 335 340 345	
ttc ttc gct ttg ggt ttg tac aac ggt act aag cca ttg tct act act	1152
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr	
350 355 360	
tct gtt gaa tct att gaa gaa act gac ggt tac tct gct tct tgg act	1200
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr	
365 370 375	
gtt cca ttc gct gct aga gct tac gtt gaa atg atg caa tgt gaa gct	1248
Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Glu Ala	
380 385 390	
gaa aag gaa cca ttg gtt aga gtt ttg gtt aac gac aga gtt gtt cca	1296
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro	
395 400 405	
ttg cac ggt tgt gct gtt gac aag ttg ggt aga tgt aag aga gac gac	1344
Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp	
410 415 420 425	
ttc gtt gaa ggt ttg tct ttc gct aga tct ggt ggt aac tgg gaa gaa	1392
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu	
430 435 440	
tgt ttc gct taa	1404
Cys Phe Ala	

&lt;210&gt; 95

&lt;211&gt; 467

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

<223> Description of Artificial Sequence: Consensus  
phytase-10-thermo-5-Q50T

&lt;400&gt; 95

Met	Gly	Val	Phe	Val	Val	Leu	Leu	Ser	Ile	Ala	Thr	Leu	Phe	Gly	Ser	-20	-15	-10	
Thr	Ser	Gly	Thr	Ala	Leu	Gly	Pro	Arg	Gly	Asn	Ser	His	Ser	Cys	Asp	-5	-1	1	5
Thr	Val	Asp	Gly	Gly	Tyr	Gln	Cys	Phe	Pro	Glu	Ile	Ser	His	Leu	Trp	10	15	20	25
Gly	Thr	Tyr	Ser	Pro	Phe	Phe	Ser	Leu	Ala	Asp	Glu	Ser	Ala	Ile	Ser	30	35	40	
Pro	Asp	Val	Pro	Lys	Gly	Cys	Arg	Val	Thr	Phe	Val	Gln	Val	Leu	Ser	45	50	55	
Arg	His	Gly	Ala	Arg	Tyr	Pro	Thr	Ser	Ser	Lys	Ser	Lys	Ala	Tyr	Ser	60	65	70	
Ala	Leu	Ile	Glu	Ala	Ile	Gln	Lys	Asn	Ala	Thr	Ala	Phe	Lys	Gly	Lys	75	80	85	
Tyr	Ala	Phe	Leu	Lys	Thr	Tyr	Asn	Tyr	Thr	Leu	Gly	Ala	Asp	Asp	Leu	90	95	100	105
Thr	Pro	Phe	Gly	Glu	Gln	Gln	Met	Val	Asn	Ser	Gly	Ile	Lys	Phe	Tyr	110	115	120	
Arg	Arg	Tyr	Lys	Ala	Leu	Ala	Arg	Lys	Ile	Val	Pro	Phe	Ile	Arg	Ala	125	130	135	
Ser	Gly	Ser	Asp	Arg	Val	Ile	Ala	Ser	Ala	Glu	Lys	Phe	Ile	Glu	Gly	140	145	150	
Phe	Gln	Ser	Ala	Lys	Leu	Ala	Asp	Pro	Gly	Ala	Asn	Pro	His	Gln	Ala	155	160	165	
Ser	Pro	Val	Ile	Asn	Val	Ile	Ile	Pro	Glu	Gly	Ala	Gly	Tyr	Asn	Asn	170	175	180	185
Thr	Leu	Asp	His	Gly	Leu	Cys	Thr	Ala	Phe	Glu	Glu	Ser	Thr	Leu	Gly	190	195	200	
Asp	Asp	Val	Glu	Ala	Asn	Phe	Thr	Ala	Val	Phe	Ala	Pro	Pro	Ile	Arg	205	210	215	
Ala	Arg	Leu	Glu	Ala	His	Leu	Pro	Gly	Val	Asn	Leu	Thr	Asp	Glu	Asp	220	225	230	

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Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr  
 235 240 245  
 Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr His Asp  
 250 255 260 265  
 Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly  
 270 275 280  
 Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Val  
 285 290 295  
 Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr  
 300 305 310  
 Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn  
 315 320 325  
 Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Val Ser Ile  
 330 335 340 345  
 Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr  
 350 355 360  
 Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr  
 365 370 375  
 Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Glu Ala  
 380 385 390  
 Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
 395 400 405  
 Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
 410 415 420 425  
 Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu  
 430 435 440

Cys Phe Ala

&lt;210&gt; 96

&lt;211&gt; 1404

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

 <223> Description of Artificial Sequence: Consensus  
 phytase-10-thermo-5-Q50T-K91A

&lt;220&gt;

95

&lt;221&gt; sig\_peptide

&lt;222&gt; (1)..(69)

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(1401)

&lt;220&gt;

&lt;221&gt; mat\_peptide

&lt;222&gt; (70)..(1401)

&lt;400&gt; 96

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Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser	
-20 -15 -10	
aca tcc ggt acc gcc ttg ggt cct cgt ggt aat tct cac tct tgt gac	96
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp	
-5 -1 1 5	
act gtt gac ggt ggt tac caa tgt ttc cca gaa att tct cac ttg tgg	144
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp	
10 15 20 25	
ggt aca tac tct cca ttc ttc tct ttg gct gac gaa tct gct att tct	192
Gly Thr Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser	
30 35 40	
cca gac gtt cca aag ggt tgt aga gtt act ttc gtt caa gtt ttg tct	240
Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Ser	
45 50 55	
aga cac ggt gct aga tac cca act tct tct gcg tct aag gct tac tct	288
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser	
60 65 70	
gct ttg att gaa gct att caa aag aac gct act gct ttc aag ggt aag	336
Ala Leu Ile Glu Ala Ile Gln Lys Asn Ala Thr Ala Phe Lys Gly Lys	
75 80 85	
tac gct ttc ttg aag act tac aat tac act ttg ggt gct gac gac ttg	384
Tyr Ala Phe Leu Lys Thr Tyr Asn Tyr Thr Leu Gly Ala Asp Asp Leu	
90 95 100 105	
act cca ttc ggt gaa caa caa atg gtt aac tct ggt att aag ttc tac	432
Thr Pro Phe Gly Glu Gln Gln Met Val Asn Ser Gly Ile Lys Phe Tyr	
110 115 120	
aga aga tac aag gct ttg gct aga aag att gtt cca ttc att aga gct	480
Arg Arg Tyr Lys Ala Leu Ala Arg Lys Ile Val Pro Phe Ile Arg Ala	
125 130 135	

96

tct ggt tct gac aga gtt att gct tct gcc gaa aag ttc att gaa ggt	528
Ser Gly Ser Asp Arg Val Ile Ala Ser Ala Glu Lys Phe Ile Glu Gly	
140 145 150	
ttc caa tct gct aag ttg gct gac cca ggt gct aac cca cac caa gct	576
Phe Gln Ser Ala Lys Leu Ala Asp Pro Gly Ala Asn Pro His Gln Ala	
155 160 165	
tct cca gtt att aac gtt att att cca gaa ggt gct ggt tac aac aac	624
Ser Pro Val Ile Asn Val Ile Ile Pro Glu Gly Ala Gly Tyr Asn Asn	
170 175 180 185	
act ttg gac cac ggt ttg tgt act gct ttc gaa gaa tct acc cta ggt	672
Thr Leu Asp His Gly Leu Cys Thr Ala Phe Glu Glu Ser Thr Leu Gly	
190 195 200	
gac gac gtt gaa gct aac ttc act gct gtt ttc gct cca cca att aga	720
Asp Asp Val Glu Ala Asn Phe Thr Ala Val Phe Ala Pro Pro Ile Arg	
205 210 215	
gct aga ttg gaa gct cac ttg cca ggt gtt aac ttg act gac gaa gac	768
Ala Arg Leu Glu Ala His Leu Pro Gly Val Asn Leu Thr Asp Glu Asp	
220 225 230	
gtt gtt aac ttg atg gac atg tgt cca ttc gac act gtt gct aga act	816
Val Val Asn Leu Met Asp Met Cys Pro Phe Asp Thr Val Ala Arg Thr	
235 240 245	
tct gac gct act caa ttg tct cca ttc tgt gac ttg ttc act cac gac	864
Ser Asp Ala Thr Gln Leu Ser Pro Phe Cys Asp Leu Phe Thr His Asp	
250 255 260 265	
gaa tgg att caa tac gac tac ttg caa tct ttg ggt aag tac tac ggt	912
Glu Trp Ile Gln Tyr Asp Tyr Leu Gln Ser Leu Gly Lys Tyr Tyr Gly	
270 275 280	
tac ggt gct ggt aac cca ttg ggt cca gct caa ggt gtt ggt ttc gtt	960
Tyr Gly Ala Gly Asn Pro Leu Gly Pro Ala Gln Gly Val Gly Phe Val	
285 290 295	
aac gaa ttg att gct aga ttg act cac tct cca gtt caa gac cac act	1008
Asn Glu Leu Ile Ala Arg Leu Thr His Ser Pro Val Gln Asp His Thr	
300 305 310	
tct act aac cac act ttg gac tct aac cca gct act ttc cca ttg aac	1056
Ser Thr Asn His Thr Leu Asp Ser Asn Pro Ala Thr Phe Pro Leu Asn	
315 320 325	
gct act ttg tac gct gac ttc tct cac gac aac act atg gtt tct att	1104
Ala Thr Leu Tyr Ala Asp Phe Ser His Asp Asn Thr Met Val Ser Ile	
330 335 340 345	

97

ttc ttc gct ttg ggt ttg tac aac ggt act aag cca ttg tct act act	1152
Phe Phe Ala Leu Gly Leu Tyr Asn Gly Thr Lys Pro Leu Ser Thr Thr	
350 355 360	
tct gtt gaa tct att gaa gaa act gac ggt tac tct gct tct tgg act	1200
Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr	
365 370 375	
gtt cca ttc gct gct aga gct tac gtt gaa atg atg caa tgt gaa gct	1248
Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Glu Ala	
380 385 390	
gaa aag gaa cca ttg gtt aga gtt ttg gtt aac gac aga gtt gtt cca	1296
Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro	
395 400 405	
ttg cac ggt tgt gct gtt gac aag ttg ggt aga tgt aag aga gac gac	1344
Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp	
410 415 420 425	
ttc gtt gaa ggt ttg tct ttc gct aga tct ggt ggt aac tgg gaa gaa	1392
Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu	
430 435 440	
tgt ttc gct taa	1404
Cys Phe Ala	

&lt;210&gt; 97

&lt;211&gt; 467

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

 <223> Description of Artificial Sequence: Consensus  
 phytase-10-thermo-5-Q50T-K91A

&lt;400&gt; 97

Met Gly Val Phe Val Val Leu Leu Ser Ile Ala Thr Leu Phe Gly Ser	
-20 -15 -10	
Thr Ser Gly Thr Ala Leu Gly Pro Arg Gly Asn Ser His Ser Cys Asp	
-5 -1 1 5	
Thr Val Asp Gly Gly Tyr Gln Cys Phe Pro Glu Ile Ser His Leu Trp	
10 15 20 25	
Gly Thr Tyr Ser Pro Phe Phe Ser Leu Ala Asp Glu Ser Ala Ile Ser	
30 35 40	
Pro Asp Val Pro Lys Gly Cys Arg Val Thr Phe Val Gln Val Leu Ser	
45 50 55	
Arg His Gly Ala Arg Tyr Pro Thr Ser Ser Ala Ser Lys Ala Tyr Ser	
60 65 70	

98

Ala	Leu	Ile	Glu	Ala	Ile	Gln	Lys	Asn	Ala	Thr	Ala	Phe	Lys	Gly	Lys	75	80	85
Tyr	Ala	Phe	Leu	Lys	Thr	Tyr	Asn	Tyr	Thr	Leu	Gly	Ala	Asp	Asp	Leu	90	95	100
Thr	Pro	Phe	Gly	Glu	Gln	Gln	Met	Val	Asn	Ser	Gly	Ile	Lys	Phe	Tyr	110	115	120
Arg	Arg	Tyr	Lys	Ala	Leu	Ala	Arg	Lys	Ile	Val	Pro	Phe	Ile	Arg	Ala	125	130	135
Ser	Gly	Ser	Asp	Arg	Val	Ile	Ala	Ser	Ala	Glu	Lys	Phe	Ile	Glu	Gly	140	145	150
Phe	Gln	Ser	Ala	Lys	Leu	Ala	Asp	Pro	Gly	Ala	Asn	Pro	His	Gln	Ala	155	160	165
Ser	Pro	Val	Ile	Asn	Val	Ile	Ile	Pro	Glu	Gly	Ala	Gly	Tyr	Asn	Asn	170	175	180
Thr	Leu	Asp	His	Gly	Leu	Cys	Thr	Ala	Phe	Glu	Glu	Ser	Thr	Leu	Gly	190	195	200
Asp	Asp	Val	Glu	Ala	Asn	Phe	Thr	Ala	Val	Phe	Ala	Pro	Pro	Ile	Arg	205	210	215
Ala	Arg	Leu	Glu	Ala	His	Leu	Pro	Gly	Val	Asn	Leu	Thr	Asp	Glu	Asp	220	225	230
Val	Val	Asn	Leu	Met	Asp	Met	Cys	Pro	Phe	Asp	Thr	Val	Ala	Arg	Thr	235	240	245
Ser	Asp	Ala	Thr	Gln	Leu	Ser	Pro	Phe	Cys	Asp	Leu	Phe	Thr	His	Asp	250	255	260
Glu	Trp	Ile	Gln	Tyr	Asp	Tyr	Leu	Gln	Ser	Leu	Gly	Lys	Tyr	Tyr	Gly	270	275	280
Tyr	Gly	Ala	Gly	Asn	Pro	Leu	Gly	Pro	Ala	Gln	Gly	Val	Gly	Phe	Val	285	290	295
Asn	Glu	Leu	Ile	Ala	Arg	Leu	Thr	His	Ser	Pro	Val	Gln	Asp	His	Thr	300	305	310
Ser	Thr	Asn	His	Thr	Leu	Asp	Ser	Asn	Pro	Ala	Thr	Phe	Pro	Leu	Asn	315	320	325
Ala	Thr	Leu	Tyr	Ala	Asp	Phe	Ser	His	Asp	Asn	Thr	Met	Val	Ser	Ile	330	335	340
Phe	Phe	Ala	Leu	Gly	Leu	Tyr	Asn	Gly	Thr	Lys	Pro	Leu	Ser	Thr	Thr	350	355	360

99

Ser Val Glu Ser Ile Glu Glu Thr Asp Gly Tyr Ser Ala Ser Trp Thr  
365 370 375

Val Pro Phe Ala Ala Arg Ala Tyr Val Glu Met Met Gln Cys Glu Ala  
380 385 390

Glu Lys Glu Pro Leu Val Arg Val Leu Val Asn Asp Arg Val Val Pro  
395 400 405

Leu His Gly Cys Ala Val Asp Lys Leu Gly Arg Cys Lys Arg Asp Asp  
410 415 420 425

Phe Val Glu Gly Leu Ser Phe Ala Arg Ser Gly Gly Asn Trp Glu Glu  
430 435 440

Cys Phe Ala

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 00/00025

## A. CLASSIFICATION OF SUBJECT MATTER

IPC7: C12N 9/16

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 9949022 A1 (NOVO NORDISK A/S), 30 Sept 1999 (30.09.99), see abstract, sequences, 100% homology --	1-14
P,X	WO 9948380 A1 (NOVO NORDISK A/S), 30 Sept 1999 (30.09.99), see abstract, sequences, 100% homology --	1-14
P,X	EP 0897985 A2 (F. HOFFMANN-LA ROCHE AG), 24 February 1999 (24.02.99), see figure 3, page 13, lines 10-11, sequences --	1-14
A	EP 0422697 A1 (AMGEN INC.), 17 April 1991 (17.04.91) --	1-14

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

4 May 2000

Date of mailing of the international search report

17-05-2000

Name and mailing address of the ISA:

Swedish Patent Office  
Box 5055, S-102 42 STOCKHOLM  
Facsimile No. +46 8 666 02 86

Authorized officer

Yvonne Siösteen/EÖ  
Telephone No. +46 8 782 25 00

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/DK 00/00025

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 9735016 A1 (NOVO NORDISK BIOTECH, INC.), 25 Sept 1997 (25.09.97), see page 10, line 22 - page 11, line 18  --	1-14
A	EP 0420358 A1 (GIST-BROCADES N.V.), 3 April 1991 (03.04.91), see page 10, line 6 - line 14 and claims 66% homology  -- -----	1-14

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 00/00025

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: **Part of claims 1 and 5**  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).:

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.  
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/DK 00/00025

In claim 1 SEQ ID NO:26 is said to be 467 amino acids long whereas in the Sequence listing it is only composed of 441 amino acids. In claim 5 SEQ ID NO:29 is said to be 1407 nucleotides long whereas in the Sequence listing it is only composed of 1404 nucleotides.

The search has been performed on the sequences as they are described in the Sequence listing.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.

PCT/DK 00/00025

Patent document cited in search report			Publication date	Patent family member(s)	Publication date
WO	9949022	A1	30/09/99	WO 9948380 A	30/09/99
WO	9948380	A1	30/09/99	WO 9949022 A	30/09/99
EP	0897985	A2	24/02/99	CN 1208768 A	24/02/99
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				NO 983364 A	25/01/99
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				AT 70537 T	15/01/92
				AT 107698 T	15/07/94
				AT 115625 T	15/12/94
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				DE 3382755 D,T	27/10/94
				DE 3382771 D,T	27/04/95
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				SE 0423845 T3	
				EP 0424990 A,B	02/05/91
				SE 0424990 T3	
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				IL 87579 A	25/01/94
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				WO 9735017 A	25/09/97

# INTERNATIONAL SEARCH REPORT

Information on patent family members

02/12/99

International application No.

PCT/DK 00/00025

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		DE 420358 T	12/10/95
		DE 69033103 D	00/00/00
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		LT 3957 B	27/05/96
		LV 10310 A,B	20/10/94
		NO 303988 B	05/10/98
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		PL 168470 B	29/02/96
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		WO 9105053 A	18/04/91

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